Bronchial hyperresponsiveness and anti-asthmatic therapy

Kraan, Jan

BRONCHIAL HYPERRESPONSIVENESS AND ANTI-ASTHMATIC THERAPY

RIJKSUNIVERSITEIT GRONINGEN

BRONCHIAL HYPERRESPONSIVENESS AND ANTI-ASTHMATIC THERAPY

proefschrift

ter verkrijging van het doctoraat in de Geneeskunde
aan de Rijksuniversiteit Groningen
op gezag van de Rector Magnificus Dr. L.J. Engels
in het openbaar te verdedigen op woensdag 25 april 1990
des namiddags te 4.00 uur.

door

Jan Kraan

geboren te Arnhem

Promotores:	Prof. dr. G.H. Koëter Prof. dr. K. de Vries
Referent:	Dr. Th.W. van der Mark
Promotiecommissie:	Prof. dr. H.J. Sluiter Prof. dr. K. Knol Prof. dr. J. Zaagsma

Ontwerp omslag:	Tom Eyzenbach
Zetwerk en lay-out:	COMPUTEKST grafische tekstverwerking, Groningen
Druk:	Dijkhuizen Van Zanten bv, Groningen

Het drukken van dit proefschrift werd financieel ondersteund door
Astra Pharmaceutica b.v. Nederland.

Aan Joke,
Lemke en Jitske

CONTENTS

PREFACE

Nearly all asthmatic patients experience shortness of breath and wheezing when they are exposed to cold air or irritants like baking fumes, exhaust gases or cigarette smoke. Salter, in 1868[1], already noticed the non-specific character of this bronchial "irritability". He stated that " .. it is clear that the vice in asthma is not the production of a special irritant, but in the irritability of the part being irritated". The clinical phenomenon that non-allergic stimuli can increase shortness of breath has been called bronchial hyperresponsiveness (BHR), which is defined as an exaggerated broncho-obstructive response following exposure to a small quantity of a non-allergic stimulus that does not provoke such a response in normal subjects[2]. BHR has been observed in patients with asthma[3-8], but also in patients with emphysema[7,9], and chronic bronchitis[10,11].

The "diseases" asthma, chronic bronchitis, and emphysema share common features such as clinical signs and pathophysiological factors and can be considered as different clinical manifestations of the same disease.

This has led to the introduction of the term chronic non-specific lung disease (CNSLD) as an overall diagnosis. For each individual patient, the diagnosis of CNSLD should be accompanied by an assessment of the following factors: 1.Complaints. 2.Degree and reversibility of bronchial obstruction. 3.Degree of bronchial hyperresponsiveness. 4.Presence, degree, and specificity of allergy. 5.Complications of CNSLD and concomitant diseases.

BHR has been considered as a characteristic phenomenon in CNSLD.

The severity of chest symptoms and the measured broncho-obstructive response following a stimulus depends on the strength of the stimulus and the degree of BHR. In a patient with a high degree of BHR, a small stimulus will suffice to cause a bronchial obstructive response. Although BHR and allergy in patients with CNSLD are independent phenomena, they influence each other in their manifestations. Therefore, the magnitude of the broncho-obstructive reaction following an allergen is not only dependent on the degree of allergic sensitization but also on the degree of BHR.

Although extensively studied, the underlying mechanism of BHR is only partly understood[2,12]. Possible mechanisms are an imbalance of autonomic nervous control, an intrinsic hyperresponsiveness of airway smooth muscle or mediator-releasing cells in the airways. Airway inflammation caused by e.g. viral infection, exposure to chemical irritants and allergic reactions is also recognized as an important modulator of BHR. There seems to be no single cause, and several mechanisms may contribute to BHR.

BHR is often "divided" into a primary part, which is probably an endogenous defect, and a secondary part, e.g. induced by exogenous factors such as allergen exposure, which is variable in time and is superimposed on the primary part. Thus, although the degree of BHR may be stable in time, there are many factors that can in-fluence it. Viral infections, exposure to allergens, and chemical irritants may induce an increase in BHR, while elimination of such factors may reduce the degree of BHR.

In hyperresponsive patients, as the term implies, an increased sensitivity can be demonstrated to a wide variety of stimuli, including pharmacological agents such as histamine, physical stimuli such as cold air, and chemical irritants such as sulphur dioxide. These non-allergic stimuli produce broncho-obstruction in nearly all asthmatic subjects. However, the cut-off point between a normal and an increased responsiveness remains arbitrary and there might be a considerable overlap between normality and disease.

The degree of BHR has been shown to be related to the severity of the disease[13] and to the amount of drug treatment required to control symptoms[14]. Furthermore it has been suggested that the degree of BHR is correlated to the progression in lung function deterioration in chronic airflow obstruction[15,16].

For these reasons, reduction of BHR with pharmacological agents can be considered an important aim in the treatment of patients with obstructive lung disease, and the effectiveness of anti-asthmatic drugs in this respect might be an important criterion of their clinical usefulness. The study of the influence of drugs on hyperresponsiveness following various triggering agents may furthermore be a tool in the study of mechanisms of BHR.

In this thesis short-term and long-term influences of some anti-asthmatic drugs on BHR are examined. The studies have been carried out in a group of young allergic patients with a reversible bronchial obstruction. The drugs used in the studies were an inhaled corticosteroid (budesonide), an inhaled beta-agonist (terbutaline), and the xanthine derivatives theophylline and enprofylline.

Bronchodilators, like beta-agonists and methylxanthines, may influence BHR mainly by a suppressive effect on bronchoconstrictor stimuli. This effect is generally of short duration. A more prolonged effect can be obtained by administration of these drugs in a sustained release form. Little is known about the effect of chronic administration of beta-agonists and methylxanthines on BHR. A protective effect of inhaled corticosteroids on BHR may possibly only occur in the long run, e.g. during maintenance treatment. Inhaled corticosteroids may decrease BHR because of a dampening effect on allergic inflammatory processes.

The bronchoprotective effect of theophylline is probably dependent on adequate plasma concentrations. This drug has, however, a narrow therapeutic range. An optimal therapeutic effect can be achieved with rather high plasma concentrations, close to the toxic range.

Additionally, theophylline has a largely unpredictable pharmacokinetic profile, which makes proper dosing for the individual patients difficult. There are some indications that the new xanthine enprofylline has more predictable pharmacokinetic properties and fewer, potentially serious, side effects, which might make the drug an alternative candidate for xanthine therapy. For these reasons, in addition to the studies of drug effects on BHR, some pharmacokinetic aspects of theophylline and enprofylline were studied.

In chapter 1, measurement of BHR by means of inhalation provocation tests and dose-response relationships of bronchoprovocative agents are described. The possible mechanisms underlying BHR are briefly reviewed, and the clinical relevance of BHR is discussed. In chapter 2, a summary is given of the literature on the treatment of BHR with various kinds of drugs and the pharmacology of the drugs that were used in our studies. Chapter 3 describes the aims of the studies in this thesis. In chapter 4, 5 and 6 the pharmacokinetic studies of theophylline and enprofylline are described. In chapter 7, 8 and 9 the

influence of drugs on BHR has been studied. Chapter 10 describes the response of sensitive parameters of bronchial obstruction, derived from maximal expiratory flow-volume curves, to maintenance treatment with budesonide.

References

1. Salter HH. On asthma: its pathology and treatment. 2nd ed. London, Churchill. 1868.
2. Boushey HA, Holtzman MJ, Shelter JR, Nadel JA. State of the art: bronchial hyperreactivity. Am Rev Respir Dis 1980; 121:389-413.
3. Tiffeneau R, Beauvallet M. Epreuve de bronchoconstriction et de bronchodilatation par aerosols. Emploi pour le depistage, la mesure et la controle des insuffisances respiratoires chroniques. Bull Acad Natl Med 1945;129:165-168.
4. Curry JJ. The action of histamine on the respiratory tract in normal and asthmatic subjects. J Clin Invest 1946; 25:785-91.
5. Townley RG, Dennis M, Itkin JH. Comparative action of acetyl-beta-methylcholine, histamine, and pollen antigens in subjects with hay fever and patients with bronchial asthma. J Allergy 1965; 36:121-37.
6. Spector SL, Farr RS. A comparison of methacholine and histamine inhalations in asthmatics. J Allergy Clin Immunol 1975; 56:308-16.
7. De Vries K, Goei JT, Booij-Noord H, Orie NGM. Changes during 24 hours in the lung function and histamine hyperreactivity of the bronchial tree in asthmatic and bronchitis patients. Int Arch Allergy 1962; 20:93-101.
8. Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. Clin Allergy 1977; 7:235-43.
9. Sluiter HJ, Orie NGM, Vegter JJM, Tammeling GJ. Emphysema and cor pulmonale. In: Bronchitis I. Orie NGM, Sluiter HJ (eds). Van Gorcum, Assen, 1961:233-46.
10. Gökemeyer JDM. Hyperreactiviteit van de luchtwegen. Thesis. Groningen, 1976.
11. Ramsdell JW, Nachtwey FJ, Moser KM. Bronchial hyperreactivity in chronic obstructive bronchitis. Am Rev Respir Dis 1982; 126:829-32.
12. Snashall PD. Mechanisms of hyperresponsiveness, general review. In: Bronchial hyperresponsiveness, normal and abnormal control, assessment and therapy. Nadel JA, Pauwels R, Snashall PD (eds). Blackwell Scientific Publications, Oxford 1987:257-314.
13. Ryan G, Latimer KM, Dolovich J, Hargreave FE. Bronchial responsiveness to histamine: relationship to diurnal variation of peak flow rate, improvement after bronchodilator, and airway calibre. Thorax 1982; 37:423-9.
14. Juniper EF, Frith PA, Hargreave FE. Airway responsiveness to histamine and methacholine: relationship to minimum treatment to control symptoms of asthma. Thorax 1981; 36:575-9.
15. Postma DS, De Vries K, Koëter GH, Sluiter HJ. Independent influence of reversibility of airflow obstruction and nonspecific hyperreactivity on the long-term course of lung function in chronic air-flow obstruction. Am Rev Respir Dis 1986; 134:276-80.
16. Taylor RG, Joyce H, Gross E, Holland F, Pride NB. Bronchial reactivity to inhaled histamine and annual rate of decline in FEV_1 in male smokers and ex-smokers. Thorax 1985;40:9-16.

CHAPTER 1

BRONCHIAL HYPERRESPONSIVENESS

Development of inhalation provocation tests

Between 1945 and 1955 Tiffeneau[1,2] and Curry[3,4] developed quantitive objective tests for bronchial responsiveness. Curry used intravenously administered histamine, which caused considerable systemic effects. Tiffeneau developed the inhalation provocation test with histamine and acetylcholine. He used the forced expiratory volume in 1 second (FEV_1) as an objective measure of bronchial obstruction, and introduced the threshold dosage as a quantitive index of hyperresponsiveness. The relation between BHR and the severity of the asthmatic condition was emphasized by Curry as well as Tiffeneau. The latter stated that the degree of bronchial sensitivity to allergens depends on (1) the degree of pulmonary allergy causing a liberation of mediators, and (2) the degree of pulmonary excitability to the mediators, the second factor being independent of allergy.

Application in clinical research

De Vries and coworkers showed that inhalation provocation tests are reproducible and that the degree of BHR has a circadian rhythm[5]. They demonstrated that the histamine threshold, repeated at the same time during the day, is reproducible within one doubling dose. Furthermore, a significant increase in BHR occurs during the night, with a maximum at about 04.00 h.

There appeared to be a correlation between the degree of BHR and the severity of bronchial obstruction[6,7]. These investigators also performed studies on the mechanisms of BHR, using provocations with fog, cold air, sulphur dioxide (SO_2), and protection with selective acting drugs[8,9,10]. When patients with obstructive lung diseases were divided into "bronchitic" and "asthmatic" patients, it was observed that the latter were more responsive to histamine, propranolol, cold air, and SO_2. The "bronchitis" group was more responsive to acetylcholine and fog. These differences in the profile of BHR between "asthmatics" and "bronchitics" suggest differences in the underlying mechanism of BHR in these patient groups[10,11]. From protection studies with specific antagonist drugs it was concluded that bronchoconstriction due to fog is predominantly caused by vagal reflex activity. SO_2-induced bronchoconstriction was best prevented by a beta-agonist[9] or by disodium cromoglycate[10], which suggests either a direct effect on smooth muscle or bronchoconstriction induced via mediator release. An important conclusion to be drawn from these studies is that a single mechanism cannot be held responsible for BHR and that underlying mechanisms might be different in certain patient groups[11].

Definition of bronchial hyperresponsiveness

The clinical phenomenon that non-allergic stimuli can increase shortness of breath has been called bronchial hyperresponsiveness (BHR) which is defined as an exaggerated broncho-obstructive response following exposure to a small quantity of a non-allergic stimulus that does not provoke such a response in normal subjects[12].

In hyperresponsive patients, as the term implies, an increased sensitivity can be demonstrated to a wide variety of stimuli, including pharmacological agents such as histamine, cholinergic agents, and propranolol[1,3,4,10], physical stimuli, such as cold air, and fog[8,13], and chemical irritants, such as SO_2, cigarette smoke, and carbon dust[14-16]. Since not all subjects with CNSLD react in the same degree to stimuli as cold air, fog, and SO_2, it is recommended that BHR should be defined more precisely with respect to the stimulus used.

Methods of measuring bronchial hyperresponsiveness

The degree of bronchial responsiveness (BR) is measured by inhalation provocation tests. The original method described by Tiffeneau was modified by de Vries et al.[5] and later by Hargreave et al.[17]. A working group on BHR of the European Society for Clinical Respiratory Physiology has proposed a standardized method for carrying out inhalation tests[18].

The methodology of inhalation provocation tests was recently reviewed[19]. Only a brief summary will be given in this thesis.

Agents

The selection of the agent for inhalation provocation depends on its applicability for inhalation, stability, and possible side effects. Although the degree of BHR to different non-specific agents generally shows, a good mutual correlation, there are sometimes differences in the responsiveness to various stimuli, e.g. in different subgroups of patients. These differences are possibly related to the variation in mechanisms that contribute to the degree of BHR in subgroups (e.g. "bronchitic" or "asthmatic")[11]. Therefore the choice of the provocative agents also depends on the purpose or reason behind the challenge.

In our studies, inhalation provocations have been carried out with histamine, methacholine and propranolol. Histamine and methacholine are widely used as bronchoconstrictor agents. Their properties have been shown to be favourable with respect to ease of preparation, long-term stability, minimal side effects, simplicity of the apparatus for inhalation provocation, good reproducibility, and a good correlation with other stimuli.

Histamine probably causes bronchoconstriction by a direct effect on histamine receptors of smooth muscle in combination with an indirect effect via vagal sensory endings and reflex activity[20]. Methacholine acts directly on bronchial smooth muscle muscarinic receptors. The bronchial obstruction caused by cholinergic agents lasts longer than histamine-induced bronchial obstruction[21]. This might be due to the clearance of the agent, or be secondary to mediator release[22].

Propranolol causes bronchoconstriction in asthmatic subjects, as opposed to normal

subjects[23,24]. There is a good reproducibility of consecutive inhalation provocation tests with this agent[25]. The mechanism by which propranolol induces bronchoconstriction has yet not been clarified. The fact that anti-cholinergic drugs have a good protective effect on propranolol induced-bronchoconstriction[26], while beta-agonists are less effective, supports the view that propranolol may act on beta-receptors in parasympathetic ganglia[27], thus enhancing parasympathetic tone. The long-lasting effect of propranolol and the protective effect of sodium cromoglycate on propranolol challenge suggest that mediator release via a non-allergic mechanism may play a role in bronchial obstruction caused by this agent[28]. Previous studies have demonstrated that changes in beta-adrenergic receptor sensitivity, e.g. caused by maintenance treatment with beta-agonists can be studied by serial bronchial challenges with propranolol[29].

Inhalation provocation tests

The bronchoprovocative solution is usually aerosolized by jet-nebulizers. The particle size produced by the nebulizer has to fall within a certain range to obtain a homogeneous deep penetration of the airways by the aerosol. The 'Wiesbadener doppelinhalator' has been characterized by Sterk et al.[30], and produces particles witha median aerodynamic diameter of 3.2 um, with a good reproducibility of the output (117±1.3 µl.min-1). The method of inhalation with a continuously generated aerosol was introduced by de Vries[5] and later modified by Hargreave[17]. This method makes it possible to deliver a precise dose of the provocating agent, if the system is calibrated at regular time intervals. For serial measurements within the same patient, it is preferable to use the same nebulizer for consecutive inhalation provocations. The aerosol is delivered for 2 minutes, at time intervals of 5 minutes. First, a control challenge is carried out with the diluent (saline). FEV_1 is then measured, and if it has not decreased by more than 10% compared with the control measurement, the procedure is continued by inhalation of consecutively increasing dosages of histamine or methacholine. The dose is increased by doubling the concentration of the agent in the solution at each dose step. The histamine and methacholine concentration range from 0.03 mg.mL^{-1} to 8 mg.mL^{-1}, concentration steps being 0.03, 0.06, 0.125, 0.25, 0.5, 1, 2, 4, and 8 mg.mL^{-1}. The effect of inhalation of subsequent aerosols with increasing concentrations is non-cumulative (histamine), or only slightly cumulative (methacholine)[31]. For practical purposes this implies that if the patient is less hyperresponsive the duration of procedure can be reduced by starting at higher concentrations of the agent.

For the study described in chapter 8 a slightly different protocol was used for histamine inhalations. In this case each concentration step was inhaled for 30 seconds, concentration steps being 1, 2, 4, 8, 16, and 32 mg.mL^{-1}. For propranolol the concentrations steps were 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 mg.mL^{-1}.

The bronchial response can be assessed by measuring FEV_1, maximal expiratory flow during partial flow-volume manoeuvres, or by measuring airway resistance. The maximal inspiration before measuring the FEV_1 influences bronchial smooth muscle tone[32], while the measurement of airway resistance by body plethysmography or by using the partial flow-volume curve is less reproducible than the FEV_1 measurement[33,34]. In our studies we have used FEV_1 as a lung function parameter. FEV_1 was measured 1 and 3 minutes after inhalation of an aerosol. The challenges were repeated until FEV_1 fell by 20% or more, compared with the baseline value.

Dosage response relationships in vitro and in vivo

In vitro The dose-response characteristics of an airway smooth muscle (ASM) preparation can be studied *in vitro* by measuring the change in length or tension in response to increasing concentrations of a constrictor agent. The (log)dose-response relationship has a sigmoid configuration (fig 1;[35]). The curve is characterized by its threshold value, slope, and the maximal response. The latter characteristic implies that at maximal doses of the

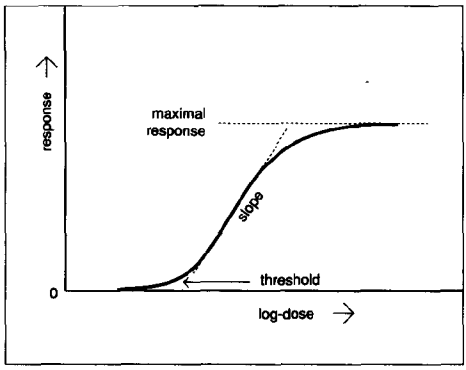

Figure 1. Dose-response relationship of airway smooth muscle *in vitro*. A smooth muscle strip is stimulated by increasing quantities of a constrictor agonist. The dose-response curve is sigmoid in shape and characterized by the threshold value, slope, and maximal response.

constrictor agent a plateau is reached. The *in vitro* responsiveness of a smooth muscle preparation is often expressed as the dose of agonist producing 50% of the maximum response.

In vivo To assess the stimulus-response relationship *in vivo* by performing inhalation provocation tests is much more complicated. Firstly, the amount of the agonist delivered at the receptor site is influenced by aerosol characteristics[36,37], pattern of breathing[36], pre-existent airway obstruction[38], and airway wall permeability[39]. Secondly, stimulated afferent nerves, neural reflexes, and inflammatory mediators that are present locally, may interact with the effect of the inhaled agent on ASM[40,41]. Following agonist-receptor interaction, smooth muscle will contract, the response being dependent on characteristics of the smooth muscle itself. The response of the bronchial smooth muscle is also dependent on muscle length-tension characteristics and factors such as transmural pressure and tissue elasticity[42]. The change in internal airway diameter following smooth muscle shortening depends on the presence of mucosal oedema or mucus secretions[43]. The change in airway resistance resulting from a certain degree of airway narrowing is dependent on initial airway narrowing. Assuming laminar flow, resistance is inversely proportional to the fourth power of the radius. Therefore a change in airway circumference leads to a greater increase in resistance, if there is a certain initial airway narrowing. The flow regime in the airway, laminar or turbulent, also has consequences for the change in airway resistance that is measured as result of the stimulus.

Although, for these reasons, the dose-response relationship *in vivo* is much more complicated, several studies have shown that, at least in normal subjects and mild asthmatics, dose-response data can be plotted as a sigmoid-shaped curve with a maximal plateau phase (fig.2;[44,45]).In more severe asthmatic patients a maximum plateau phase cannot be

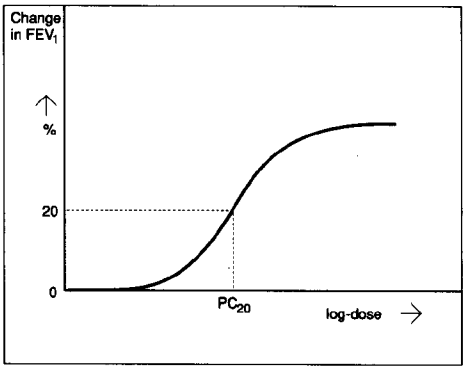

Figure 2. Airway dose-response curve. *In vivo*, the airway is stimulated by increasing doses of an agonist, administered by inhalation. The response is measured as a change in a lung function parameter such as the FEV_1. Airway responsiveness is often expressed as the PC_{20} which is the provocation concentration of the agonist that is required to produce a fall in FEV_1 of 20%.

reached, because of excessive airway narrowing at submaximal doses. The curve is then better described by a second-degree polynomial[46]. Single parameters derived from the dose-response curve are generally used to express the degree in hyperresponsiveness. The most widely used parameter is the concentration of bronchostrictor causing a fall of 20% in baseline FEV_1 (PC_{20}).Other parameters are the threshold value, the maximal slope, and the maximal response[19]. The threshold value and PC_{20} value describe the position of the curve in the coordinate system. Both can be derived from a least-square fitting of the curve to the experimental data. The calculation of the PC_{20} value by linear extrapolation is a widely used procedure. Theoretically this procedure may not be entirely correct, because of the curvilinearity of the dose-response curve[46]. The threshold value, calculated from the variability of the baseline value measurement (-2SD) is less reproducible than the PC_{20} value[47]. The reliability of the calculation of the slope of the dose-response curve is limited, because the data points are found within a limited range. The usefulness of the maximal response as a parameter of hyperresponsiveness is limited by the fact that it can only be measured in normal and in mild hyperresponsive subjects[44].

The PC_{20} is therefore the best documented and most reproducible parameter derived from the dose-response curve. It discriminates best between subjects with asthmatic symptoms and normal subjects[31].

Analysis of changes in airway smooth muscle responsiveness in vitro and bronchial responsiveness in vivo

Changes in ASM responsiveness *in vitro* can be analysed by means of dose-response curves (fig 3). Curve A shows a normal dose-response relationship. Increased ASM responsiveness may be due to a leftward shift of the curve (curve B[48]). It is a result of increased response of ASM receptors to the constrictor agonist, due to e.g. increased receptor affinity, increased receptor density, or increased access to the receptor through tissue barriers. Curve C represents an increase in maximal response. It may be due to post-receptor changes in the smooth muscle itself or to changes in cell-to-cell coupling.

Changes in BR *in vivo* can be analysed in a way analogous to ASM reactivity *in vitro*[42]. However, the change in airway narrowing due to a certain degree in ASM shortening is also dependent on the proportion of ASM present in the airway circumference, and on the (internal) airway wall thickness[42,43]. Thus, in addition to ASM hyperresponsiveness, there

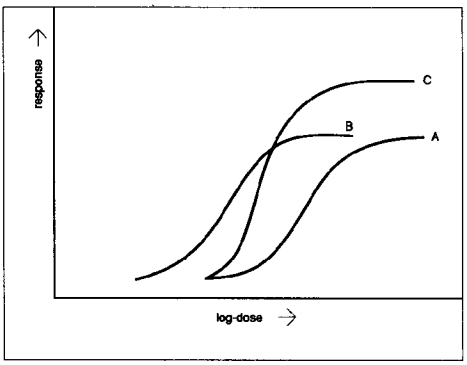

Figure 3. Changes in airway smooth muscle responsiveness *in vitro*. A. normal curve. B. Increased responsiveness caused by parallel left-ward shift of the curve. C. Increased responsiveness caused by an increase in the maximal response.

are two other important factors which may lead to increased maximal airway narrowing: smooth muscle hypertrophy and increased airway wall thickness due to submucosal oedema or airway secretions.

Unfortunately, it is often impossible to obtain complete dose-response curves *in vivo*, including threshold value and maximal response, which makes it impossible to determine whether changes in BHR *in vivo* are due to a parallel shift of the dose-response curve or a change in maximal response (fig.4[49]).

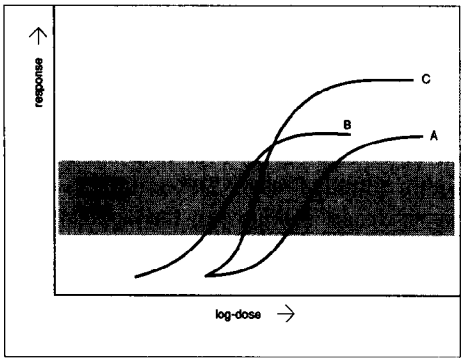

Figure 4. Changes in bronchial responsiveness. Curves A, B, and C as in figure 3. With *in vivo* measurement, it is not possible to obtain complete dose-response curves of airway smooth muscle, including threshold and maximal response. Therefore, the nature of increased responsiveness (deviation supersenstivity (curve B) or non-deviation supersensitivty (curve C)) cannot easily be analysed.
(Adapted from NC Thomson and RS Roberts: ref.49)

Mechanisms causing bronchial hyperresponsiveness

Although extensively studied,the mechanisms of BHR are still not completely understood[12,50,51]. Probably no single causative defect underlies the phenomenon of BHR. Different mechanisms may be operative at the same time and may influence each other. Subjects with CNSLD may not always react in the same degree to certain pharmacological and chemical stimuli. It has been noted that patients with predominantly asthmatic characteristics tend to be more responsive to SO_2, propranolol, and cold air while patients of the chronic bronchitis type tend to be more responsive to fog. These differences in BHR profiles suggest that different mechanisms may predominate in different subgroups of CNSLD patients[52].

Factors that may be involved in BHR are airway caliber, imbalance in autonomic control, intrinsic abnormalities in airway smooth muscle or mediator releasing cells, and inflammatory changes in the airways.

BHR may be subdivided in a primary part,which is probably genetically determined[53,54], and an acquired secondary part which is influenced by exogenous factors such as exposure to allergens[10], chemical irritants[55], or (viral) airway infections[56]. BHR can be stable over long periods of time[57]. On the other hand, the withdrawal of exogenous factors[58,59] or treatment with anti-asthmatic drugs[60] may decrease BHR.

Airway calibre

In subjects with BHR there is a relationship between baseline airway calibre and the degree of responsiveness. Subjects with a larger degree of bronchial obstruction tend to be more responsive[61,62,63]. This relationship is in general rather weak and the correlation between bronchial obstruction and BR seems to be different in "asthmatics" and in "bronchitics"[62,63]. Asthmatic patients tend to be more responsive to an inhaled stimulus than bronchitics having the same degree of bronchial obstruction. Furthermore, a much increased BR can be observed in asthmatic subjects with normal baseline lung function[61].

Although both bronchial obstruction and BHR might be result of the same underlying mechanisms, it is often stated that a decrease in baseline airway calibre will lead to an increased response to a constrictor agent. Airway resistance is dependent on airway diameter. The same absolute amount of airway smooth muscle shortening causes a larger increase in airway resistance in an airway narrowed by preconstriction than in a dilated airway. It is, however, unknown whether a certain stimulus will cause the same absolute amount of muscle shortening in a narrowed and a dilated airway (fig 5;[64]). Due to changes

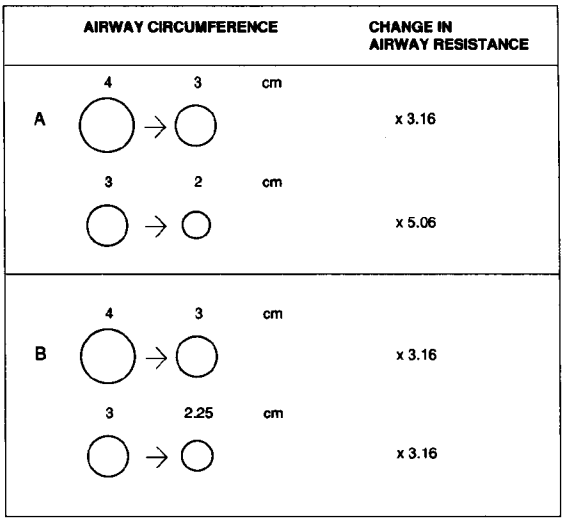

Figure 5. Change in airway resistance caused by a bronchoconstriction. A. The same absolute reduction (1 cm) in airway circumference in a normal airway and an airway already narrowed by prior constriction. B. The same percentual reduction (25 %) in airway circumference in a normal airway and an airway already narrowed by prior constriction. (Adapted from AE Tattersfield:ref 64.)

in preload for the contracting muscle, the amount of shortening might decrease if the airway is already constricted. If, for example, a certain stimulus in a narrow and dilated airway would lead to the same percentage of shortening, instead of the same absolute amount, the PC_{20} calculated for both situations would be equal. Therefore prior bronchoconstriction cannot be simply brought forward as a cause of increased BR. However, the dose-response curve of ASM *in vitro* and *in vivo* shows a leftward shift after prior constriction and this is probably caused by synergy of increased contractility (which caused the prior constriction) and the bronchoconstrictive stimulus[42]. In addition, more central deposition of the inhaled aerosol in the presence of bronchial obstruction may cause an increased response[38].

In contrast to prior smooth muscle constriction, increased mucosal thickening or obstruction of the airway lumen by secretions is probably an important geometrical cause of an increased BR. The smaller airway diameter due to inflammatory processes in the airway wall may magnify the effect of smooth muscle contraction[43]. In consequence the dose-response curve measured as airway resistance or FEV_1 has an increased slope and an increased maximal response. Because pathological changes, such as submucosal thickening, are generally found in asthma[65], this factor may be an important cause of increased BHR. Moreover, under such circumstances increased BR does not necessarily reflect an increased bronchial smooth muscle responsiveness[66].

Disturbances of autonomic regulation

An imbalance in neural or neurohumoral control mechanisms has long been considered an important factor in causing BHR. In recent years it has become clear that the autonomic control of the airways is much more complex than was previously thought. In addition to cholinergic contracting mechanisms and adrenergic relaxing mechanisms, a non-cholinergic excitatory neural pathway and a non-adrenergic inhibitory pathway are present, and abnormalities in each of these systems may play a role in BHR[27,67].

Cholinergic mechanisms Neural bronchoconstrictive stimulation in the airways is predominantly mediated via the parasympathetic nervous system[68]. Afferent airway nerves stimulated via c-fibre endings and irritant receptors excite efferent parasympathetic nerves, running in the vagal nerve. These efferent nerves terminate in synapses in airway wall ganglia to post-ganglionic nerves that innervate ASM and mucous glands, acetylcholine being the neurotransmitter at these sites[69].

Many agonists that produce bronchoconstrictor symptoms in asthmatic subjects such as fog, carbon dust, SO_2, ozone, and inflammatory mediators, stimulate vagal sensory nerve receptors, and the effects of the agents can be inhibited by anticholinergic drugs[40,55,70,71]. The mechanism by which increased cholinergic activity plays a role in BHR is unknown. There is some evidence that increased cholinergic activity may be present as a primary phenomenon at least in subgroups of patients with CNSLD[72].

The discovery of various different muscarinic receptor subtypes has given rise to new hypotheses about possible dysfunctioning of cholinergic pathways. Besides the M_3 subtype receptor on smooth muscle cells, muscarinic receptors of the M_2 subtype are present on the presynaptic membrane of postganglionic cholinergic fibres[73]. Activation of these receptors inhibits the release of acetylcholine. Via these receptors a negative feed-back might control

increased cholinergic activity. In asthma these receptors might be dysfunctional, which could give rise to increased acetylcholine release.

There is, however, also evidence that increased cholinergic activity may be caused by interaction with inflammatory processes, e.g. caused by irritants. Ozone inhalation[55] and viral infection[56] of the respiratory tract lead to increased BHR which can be inhibited by anticholinergic drugs. Three possible ways of interaction of inflammatory processes with autonomic nerve control have been proposed. A. Increased afferent nerve activity, caused by damage of airway epithelium which facilitates access to nerve receptors[65,74]. Moreover, irritants may stimulate airway epithelium to produce inflammatory mediators[75,76]. B. Inflammatory mediators or neuropeptides might facilitate efferent cholinergic pathways[40] C. Mediators may increase the sensitivity of airway smooth muscle to cholinergic agents[41,77].

Adrenergic mechanisms Adrenergic control mechanisms of the airways consist of sympathetic nerves supplying the airways, and circulating catecholamines. The fact that beta-blocking agents induce bronchoconstriction in asthmatic subjects supports the view that adrenergic mechanisms play an important role in controlling ASM tone in these patients[23,24].

Sympathetic nerves are sparse in human airways. There is no direct adrenergic nervous supply of human ASM. Most nerves are related to mucous glands and airway ganglia[78,79]. It has been demonstrated that transmission of nerve activity in cholinergic ganglia can be inhibited by sympathetic nerves[80,81]. Circulating catecholamines, produced by the adrenal medulla, probably play an important role in regulating ASM tone in asthmatics. Although these circulating cathecholamines probably have a direct effect on ASM cells, they also might have an inhibiting effect on the transmission of nervous activity through cholinergic ganglia via beta-receptors located at these ganglia[27]. This is supported by the observation that bronchoconstriction induced by a beta-blocker in asthmatics can be more effectively reversed by an anticholinergic drug than by a beta-agonist[82]. This suggests that a beta-blockade may enhance neural transmission through cholinergic airway ganglia.

Decreased secretion of epinephrine from the adrenal gland might lead to impaired protection against bronchoconstriction. There are some indications in the literature that epinephrine secretion is impaired in asthmatic patients, especially in situations in which normally an increased secretion is observed[83,84,85].

Beta-receptor function Impaired metabolic responses to cathecholamines in vivo[86,87], and impaired beta-adrenergic responses in vitro[88,89] have frequently been observed in asthmatic subjects. This has led to the hypothesis that a primary defect in beta-receptor function might underlie asthma and BHR[90]. However, from further research it has become clear that this impairment is not a primary factor[29]. Beta-receptor subsensitization or tachyphylaxis, as the phenomenon has been called, may be secondary to external factors, such as viral infections[91], allergen exposure[92], and maintenance treatment with beta-adrenergic drugs[93]. It has been shown that a decrease in leukocyte beta-receptor density, as well as a decreased post-receptor responsiveness to isoproterenol in vitro, develops after allergen challenge in vivo[92,94]. Such a decrease in beta-adrenergic function might also occur in ASM, as is suggested by the finding of increased BR to an inhaled beta-blocker, propranolol, after allergen challenge[92].

Maintenance treatment with beta-agonists and tachyphylaxis Beta-agonists are widely used in the treatment of obstructive lung diseases. It has been shown that prolonged stimulation of beta-receptors by their agonists in vitro can induce a decrease in responsiveness to these agonists. This phenomenon, called tachyphylaxis, has been demonstrated in tissues such as peripheral blood leukocytes and isolated ASM[93,95,96]. Tachyphylaxis has also been demonstrated in vivo in normal airways after prolonged treatment with oral and inhaled beta-agonists[97,98]. In asthmatics, some studies have shown a development of beta-adrenergic tachyphylaxis leading to impaired bronchodilator responsiveness[99,100], though this was not found in other studies[98,101].

In general, evidence exists that both in normal and in asthmatic subjects, tachyphylaxis can be induced by maintenance treatment with beta-agonists[102]. Although this may lead to a decrease in bronchodilator responsiveness, the overall bronchodilator responsiveness to beta-agonist treatment seems to be well-preserved. Therefore the clinical relevance of this phenomenon remains to be established. It is possible that in a severe asthmatic state there is an impaired beta-receptor function induced by a combination of factors, e.g. viral infection, allergen exposure, and overuse of (inhaled) beta-agonists, leading to non-responsiveness to beta-agonist drugs.

Since an impaired function of the beta-adrenergic system might be a causal factor for BHR, it may be important to study the effect of maintenance treatment with beta-agonists on BHR. Data from the literature on this subject will be discussed in chapter 2.

Non-adrenergic non-cholinergic pathways In addition to the adrenergic and cholinergic neural pathways, non-adrenergic non-cholinergic (NANC) nerves have been demonstrated in animals and humans[67]. Furthermore, a number of neuropeptides related to these NANC-nerves has been identified[103]. The potential effects of these neuropeptides on airway tissue are very relevant to the pathology of asthma.

Non-adrenergic inhibitory pathways have been functionally demonstrated in human airway tissue *in vitro*[104], and in animals *in vivo*[105]. In humans, these NANC-nerves constitute the sole bronchodilatory nervous pathway that directly innervates ASM. The possible neurotransmitters of this system, vaso-intestinal peptide (VIP) and peptide histidine-methionine (PHM), are very potent smooth muscle relaxants[106]. VIP has been localized to nerves and ganglia supplying human airways[103], and VIP-receptors have been demonstrated auto-radiographically on airway smooth muscle in large, but not in small airways[107]. NANC-nerves run via the vagal nerve and synapse in the airway wall. There is no evidence for a dysfunction of the NANC inhibitory system as a primary factor in BHR. It has been hypothesized that a decrease in the function of this inhibitory system may develop in asthma if neuropeptides such as VIP and PHM are degraded by enzymes that are released by inflammatory cells[27].

A non-cholinergic excitatory neural effect in animal[108] and human[109] airway tissue has been demonstrated. This effect can be mimicked by substance P, which makes it a likely mediator. The presence of substance P and other structurally related tachykinins, such as neuropeptide K, in the human lung has been demonstrated by immunoreactivity studies on afferent nerves and ganglia[110]. Substance P is a potent bronchoconstrictor of airway smooth muscle *in vitro*, but is less potent after infusion *in vivo*[111]. Substance P induces mucosal oedema and extravasation of plasma, and has been shown to stimulate airway

mucous secretion and mediator release *in vitro*[112]. Therefore tachykinins may have actions that are very relevant to the pathophysiology of asthma. It is unknown whether an increased activity of the NANC excitatory pathway plays a role in BHR. Airway mucosal damage might expose afferent nerve (C-fibre) endings to the irritant effect of inflammatory mediators. Stimulation of these afferents might lead to a local axon reflex with local release of neuropeptides such as substance P, which act directly on smooth muscle, microvasculature,[110] or mucous glands, or indirectly via effects on ganglia or postganglionic nerves[113].

Inflammatory processes

In recent years, airway inflammation has received much attention as a causal factor for BHR[39,114,115,116]. The pathology of severe asthma is characterized by loss of epithelial cells, basal membrane thickening, infiltration of mucosa and submucosa by neutrophils, eosinophils and mononuclear cells, and by ASM hypertrophy[65]. Bronchial biopsies taken from mild asthmatic patients have sometimes shown severe epithelial destruction[74].

Evidence for a relationship between inflammation and BHR has been based on the observation that an increase in BR may occur after inhalation of agents that can induce airway inflammation, such as ozone[55], nitrogen dioxide[117], allergen[118], airway viral infections[56], toluene diisocyanate[119] and western red cedar[120].

Mediators and inflammatory cells in asthmatic reactions In allergic asthmatic patients, allergen exposure may lead to a late bronchial obstructive reaction (late asthmatic reaction (LAR)), occurring about 3 hours after the early asthmatic reaction (EAR). The LAR reaches its peak about 8 hours after the challenge, and may last for more than 24 hours[121].

Bronchial obstruction during the EAR is mainly caused by the direct bronchoconstrictor effect of mediators such as histamine, prostaglandin D_2 (PgD_2)[122], leukotriene C_4 (LTC_4), and leukotriene D_4 released by mast cells. This bronchoconstriction can be inhibited or reversed by bronchodilators.

The LAR reacts very poorly to bronchodilators[123], but pretreatment with inhaled or oral corticosteroids prevents its occurrence[121,124]. It has been shown that the LAR is preceded and accompanied by increased numbers of eosinophils in peripheral blood[125], and an increase of eosinophils and neutrophils in bronchoalveolar lavage fluid[126,127], and airway submucosa[128]. Mediators like eosinophil chemotactic factor[129], neutrophil chemotactic factor[130], leukotriene B_4 (LTB_4)[131], and platelet activating factor (PAF)[132] (probably released by mast cells), may be responsible for the chemo-attraction of these cells. Some of these mast cell mediators are also responsible for increased vascular permeability and stimulation of mucosal glands.

There is increasing evidence that airway macrophages, monocytes, and lymphocytes, activated in the allergic process, release mediators such as interleukins and gamma-interferon, which may secondarily activate other inflammatory cells[127].

Apart from an accumulation of inflammatory cells in the airways, there is also evidence for activation of these cells. After allergen inhalation granulocytes and monocytes have increased numbers of complement receptors[133], and an increased cytotoxic activity[134]. Furthermore, an increased concentration of eosinophil secretory products in the sputum and BAL fluid is found during the LAR[126], but also in stable asthma[135]. The occurrence

of these substances in the airway lumen may be causally related to the mucosal damage seen in asthmatic patients.

Airway infiltration of neutrophils has been demonstrated after inhalation of ozone[136]. Neutrophil as well as eosinophil infiltration also occurs after toluene diisocyanate inhalation (TDI)[137], whereas after western red cedar inhalation eosinophils predominate[138].

Relationship of bronchial hyperresponsiveness to inflammatory changes After it had been shown that allergic patients may develop an increase in BHR after an allergic broncho-obstructive reaction[10], it was shown that this phenomenon is dependent on the presence of the LAR. After the lung function disturbance due to the LAR had disappeared, the increase in BR could be demonstrated up to at least 2 weeks after the allergen challenge[118]. A persistent increase in BR might be due to chronic exposure to allergens like house dust mite[139] or seasonally occurring allergens.

The importance of inflammatory changes for the subsequent development of increased BR has been studied experimentally in several ways. Studies in which rabbits were challenged with allergens after depletion and successive repletion of granulocyte cells have shown the importance of the presence of these cells for the occurrence of the LAR[140]. In dogs challenged with ozone the increase in BHR was closely related in time with the increase in the number of neutrophils and airway epithelial cells in the BAL fluid[136]. In a guinea-pig study, the increase in BR occurred before the influx of neutrophils. Changes that coincided with increased BHR were submucosal oedema, and a decrease in the number of goblet cells[140]. In some allergen challenge studies in humans, the increase in BR already occurred before the LAR[141,142], perhaps at a time when an increased number of inflammatory cells is already present[126].

It is unknown how the inflammatory processes described above are related to increases in BR that occur after exposures to allergens, chemical irritants and sensitizers. Airway inflammation may increase BR for geometrical reasons: mucosal thickening due to oedema may increase the effect of a certain degree of smooth muscle shortening on airway resistance[43]. Inflammatory mediators may increase BR, such as has been demonstrated for inhaled LTB_4[143], PgD_2[41], thromboxane mimetics[144], and PAF[145]. The mechanism behind this is unknown. Inhalation of LTB_4 led to an increase of the thromboxane B_2 concentration in BAL fluid in dogs, whereas administration of a thromboxane synthesis inhibitor before inhalation of LTB_4 prevented the increase in thromboxane concentration as well as the expected increase in BR[143]. The increase in BR after ozone, allergen, or PAF inhalation can also be prevented with a thromboxane synthesis inhibitor[144,146]. Mediators such as thromboxane B_2, PgD_2, and serotonine[40] may facilitate neurotransmission in cholinergic ganglia, or increase ASM responsiveness to cholinergic action.

Many mediators of inflammation cause increased microvascular permeability to plasma proteins. These mediators probably have a contractile effect on endothelial cells of post-capillary venules, which may lead to submucosal oedema and to leakage of protein rich fluid through the airway epithelium[147,148]. These events occur rapidly and may form an explanation for the early occurrence of increased BHR after exposure to inflammatory agents[140142].

Epithelial damage may cause better access to afferent receptors, leading to increased cholinergic activity or axon reflex activity. Loss of an epithelium-derived relaxant factor might also play a role[149].

An intriguing question several researchers have posed is why in some clinical situations and experimental models airway inflammation is present without increased BR. Kerrebijn et al. found that asthmatic patients were far more hyperresponsive than patients with cystic fibrosis, despite overt airway inflammation in the latter[150]. In an animal model of airway inflammation caused by endotoxin exposure, Pauwels et al. found that some inbred animal strains showed no increased BR in contrast to other strains, despite overt airway inflammation[151]. Thus, although airway inflammation appears to be involved in both induced and stable BHR, it is not known which inflammatory cells or mediators are responsible in the first place.

Intrinsic abnormalities in airway smooth muscle

A number of studies have compared *in vitro* to *in vivo* airway smooth muscle responsiveness[152,153,154]. Generally, a wide range of BR *in vivo* was found, while there was a relatively narrow range in responsiveness *in vitro* and no correlation was found between *in vivo* and *in vitro* responsiveness. Most of these studies were carried out in patients with obstructive lung diseases, and relatively mild BHR.

Probably the characteristics of BHR in chronic airflow obstruction are different from those in asthma: the correlation between BHR and the degree of airflow obstruction is close in chronic airflow obstruction, while it is poor in asthma[61-63]. Furthermore, BHR is often more severe in asthmatic subjects if subjects with the same degree of airflow obstruction are compared.

In a study of de Jongste and coworkers, ASM responsiveness as measured by the maximal isometric tension in response to histamine was higher in subjects with obstructive lung disease than in normal subjects. However responsiveness to methocholine was equal in both groups[155]. In two studies of *in vitro* ASM responsiveness of asthmatic subjects, greatly increased responses to histamine, methacholine and LTC_4 were found[156,157]. A study using dogs allergic to ovalbumin[158] suggested that allergic inflammatory processes may be causally related to increased ASM responsiveness. In this study it was found that isotonical maximal shortening of tracheal smooth muscle was increased after allergen exposure.

The cause of increased ASM responsiveness in asthma is unknown, but it may result from smooth muscle hypertrophy, changes in cell-to-cell coupling, or changes in ASM post-receptor mechanisms.

Intrinsic abnormalities of mediator releasing cells

An increased ability of leukocytes to release histamine, oxygen radicals, and LTC_4 has been observed in asthmatic subjects[159-162]. This increased releasability was significantly correlated to the degree in BHR[159-161]. These observations suggest an association between asthma and increased releasability of leukocytes. The significance of this association remains to be established. It might indicate that the hyperreleasability of mediator-producing cells influences BHR. On the other hand, the association of these phenomena might be an expression of a common factor underlying the hyperresponsiveness of mediator releasing cells, and, for example, ASM cells.

Bronchial hyporesponsiveness

Normal subjects and mild asthmatic subjects show a plateau, i.e., a maximal response, on histamine or methacholine, while in more severe asthmatic subjects the maximum value of the curve cannot be demonstrated, because a severe decrease in airway calibre is already seen at submaximal doses[44,45].

The fundamental mechanisms of BHR can be investigated using a different approach, i.e.by first studying the mechanism that is responsible for the limited responsiveness (bronchial *hypo*responsiveness) in normal subjects. An impairment in inhibitory mechanisms might then lead to BHR[163]. Sterk et al. concluded from their studies that the limited BR in non-asthmatics is not due to airway dilatation following lung inflation, changes in adrenergic, cholinergic, or ganglion-transmitted non-adrenergic inhibitory mechanisms, nor to the release of bronchodilatory prostaglandins[45,164].

It has been suggested that in normal subjects important airway narrowing by constriction of ASM is prevented by viscous and elastic loads which support elements of the airway wall and surrounding tissues[163]. Abnormalities in these supporting tissues, caused for example by inflammatory processes, might make it possible that quasi-isotonic contraction of ASM occurs in asthmatic subjects[42].

Hereditary factors

Several studies have demonstrated that genetic factors may cause a susceptibility to develop BHR: among non-symptomatic relatives of patients with asthma, an increased incidence of BHR has been found[165,166]. However, the fact that in population studies the distribution of BR is unimodal lognormal[167,168] suggests that besides genetic factors a multiplicity of other (exogenous) factors plays an important role.

Clinical relevance and diagnostic value of bronchial hyperresponsiveness

Tiffeneau was the first to recognize the relationship between the severity of asthma and the degree of BHR[2]. This observation has been confirmed by other studies[17,169]. The degree of BHR is correlated to the bronchial responsiveness following cold air inhalation and to the degree in exercise-induced bronchoconstriction[170,171].The higher the responsiveness to histamine or methacholine, the greater the lung function disturbance in the early morning and the diurnal variation in peak flow rate[17], and, in allergic subjects, the greater the sensitivity to inhaled allergens[2,10,172]. Finally, the degree in BHR is related to the minimum medication required to control symptoms[173]: the lower the PC_{20}, the greater the amount of treatment required. Therefore measurement of BHR is of great value in the estimation of the severity of the disease as well as in the assessment of the response to therapy.

The validity of the BHR measurement in establishing a diagnosis of asthma or CNSLD has been questioned[174]. Cockcroft et al.[17] studied asthmatic patients, as diagnosed from a history of current or past asthmatic complaints, with subjects without a history of chest symptoms or past airway disease. A histamine PC_{20} below 8 $mg.mL^{-1}$ was found in 97% of the asthmatic subjects, while all control subjects had a histamine PC_{20} above 8 $mg.mL^{-1}$. Cockcroft et al. proposed a PC_{20} value of 8 $mg.mL^{-1}$ (or a range between 4 and 16 $mg.mL^{-1}$) as a diagnostic cut-off point for the presence of BHR. In their study a group of

patients with rhinitis without lower airway symptoms and patients with cough or vague chest symptoms appeared to have histamine PC_{20} values below as well as above 8 mg.mL^{-1}. If the hyperresponsive PC_{20} values in the latter group are considered as false positive findings, the sensitivity and specificity of the test would be 97% and 71%; it is clear that the predictive value of BHR measurement would be strongly dependent on the constitution of the population under study[174].

In a study of Ramsdale et al., patients with rhinitis (without asthmatic symptoms) who had a histamine PC_{20} below 8 mg.mL^{-1} appeared to have increased diurnal variations in peak flow rate, increased sensitivity to cold air inhalation, or both[175]. These subjects might thus be considered to have subclinical asthma, which went unnoticed due to, e.g., low levels of daily exercise or poor perception of bronchial obstruction; therefore the low histamine PC_{20} values in these subjects should be considered as indicative of BHR.

There are indications that the degree in BHR may be related to the prognosis of asthmatic children and the progress of chronic airflow limitation[176,177,178]. If this is the case, the importance of therapeutic interventions aimed at decreasing BHR goes beyond the control of current symptoms[179].

Assessment of bronchial obstruction

Bronchial obstruction can be estimated by measuring airways resistance or by measuring airflow during forced expiratory manoeuvres.

Airway resistance (Raw) is measured at normal tidal breathing or during panting, usually by means of a body plethysmograph. In normal subjects the major site of airway resistance is in the larger airways, whereas in subjects with CNSLD, medium-sized and smaller airways contribute more to airways obstruction[180]. Airways resistance depends on the lung volume at which it is measured. The reciprocal of airways resistance divided by the lung volume,the specific airways conductance (sGaw) is approximately independent of the lung volume. Airways resistance is a very sensitive measurement of airways narrowing. The test is not effort-dependent. The reproducibility is relatively poor, but strongly dependent on technical factors. The intra-individual coefficient of variation of Raw is 10-20%[34] and of sGaw 7-9%[181,182].

During a forced expiration, flow is mainly dependent on the frictional resistance of smaller airways, on the elastic recoil pressure of the lung, on elastic properties of the airways, and on gas density and viscosity. During a maximal expiratory manoeuvre an expired volume may be measured against time, such as FEV_1, or expiratory flow against remaining lung volume: the maximal expiratory flow-volume curve (MEFV-curve). During the first part of the expired volume (approximately 25%) the flow is effort dependent, and during the final part it is effort-independent. The FEV_1 measured with a wet or dry spirometer is a simple and very reproducible measurement (variation coefficient 3-8%)[33], but less sensitive than other parameters such as airways resistance. MEFV-curves are obtained by measuring flow as a function of lung volume. Flow can be measured by means of a pneumotachograph or by differentiating the volume signal of a spirometer. Ideally, flow measurements from serially obtained curves refer to the same absolute lung volume. In practice the forced expiratory vital capacity is used as a reference lung volume.

Expiratory flows at lower lung volumes such as the maximal expiratory flow at 50% and 25% remaining volume ($\dot{V}_{E, max\ 50}$ and $\dot{V}_{E, max\ 25}$ resp.) are probably more sensitive parameters of bronchial obstruction than the FEV_1. These parameters are considered to reflect small airways obstruction more specifically. The intra-individual variation coefficient is reported as 10% before, and 16% after bronchoconstriction[183].

The deep inspiration which precedes maximal expiratory manoeuvres influences airway caliber. In normal and mild astmatic subjects it leads to an increase in airway caliber, while in more severe asthmatics it leads to bronchoconstriction. To avoid these problems, partial expiratory flow volume curves have been recommended. These curves start at 60% of the vital capacity. The intra-individual coefficient of variance of this sensitive test of airways obstruction is reported as 11% before, and 21% after bronchoconstriction[183].

Bronchial obstruction in asthmatic patients is generally thought to be determined by bronchial smooth muscle contraction and by inflammatory processes in the bronchial wall[65]. MEFV curves are generally thought to give additional information about the severity of bronchial obstruction[184,185]. On the basis of wave-speed mechanics, Dawson and Elliott[186] came to the conclusion that the flow-limiting segment moves towards the peripheral airways with decreasing remaining lung volume. Measuring flows at different lung volumes may therefore give an approximate indication of the site of airway obstruction.

In clinical practice the MEFV-curves are usualy analysed in terms of maximum flows at a given volume, and often qualitatively interpreted with regard to the shape of the curve. Even in patients with a mild bronchial obstruction[187,188] the flow-volume curves are more bowed towards the volume axis.

The exact pathophysiological basis of increased curvilinearity of the MEFV-curve is unknown and the question arises whether this increased convexity of the flow-volume curves reflects a specific pathological process that may eventually be influenced by treatment. Many authors are of the opinion that the decrease in expiratory flow at the lower end of the MEFV-curve, i.e., near residual volume, may be caused by preferential obstruction of the peripheral airways[189,190].

Mead has developed the concept of inhomogeneous emptying of the lung during a forced expiration and has shown on theoretical grounds that in case of such an inhomogeneity the flow-volume curve should be convex towards the volume axis[191]. It is therefore possible that the increased curvilinearity of the MEFV-curve is caused by regional inhomogeneity of forced expiratory flow, i.e. the existence of regions with a different time constant (flow divided by volume) in the lung[191].

Summary

Bronchial hyperresponsiveness is both a central clinical phenomenon and probably also a fundamental abnormality in CNSLD. BHR can be quantified by inhalation challenge tests with bronchoconstrictive stimuli. These tests are extensively used in clinical practice and for research purposes.

Inhalation challenge methods have recently been standardized, and are most often carried out with histamine and methacholine. These agents are inhaled either from a continuously generated aerosol or from a breath-driven dosi-meter aerosol. The bronchocon-

strictive response is measured by the FEV_1 or, in the case of specialized research purposes, by airway resistance or partial flow-volume curves. The PC_{20} is the best documented and most reproducible parameter derived from the dose-response curve. It discriminates best between subjects with asthmatic symptoms and normal subjects.

Although extensively studied, the mechanisms of bronchial hyperresponsiveness are still not completely understood. Probably no single causative defect underlies the phenomenon of BHR. Different mechanisms may be operative at the same time and may influence each other. BHR may be subdivided in a primary part which is probably genetically determined and an acquired secondary part which is influenced by exogenous factors such as exposure to allergens, chemical irritants, or (viral) airway infections. BHR can be stable over long periods of time. On the other hand, the withdrawal of exogenous factors, or treatment with anti-asthmatic drugs may decrease BHR.

Abnormalities in autonomic regulation, such as an increased cholinergic reflex acivity or impairments in the beta-adrenergic system may play an important role in BHR. Abnormal activity of non-cholinergic or non-adrenergic neural pathways may also play a role. It is not clear whether such abnormalities are primary factors or whether other processes such as airway inflammation may lead to abnormalities in the autonomic regulation of ASM tone.

In recent years inflammatory processes have received much attention as a causal factor for BHR. Evidence for a relationship between inflammation and BHR is based on the observation that after exposure to agents that may induce airway inflammation, such as allergens or chemical sensitizers or after viral infections, an increase in BHR may occur. The relation between airway inflammation and BHR is not exactly known. One factor that almost certainly plays a role is the increase in airway wall thickness by oedema or secretions that decrease the airways lumen.
There is evidence, at least in asthmatic subjects, of an increased responsiveness of ASM *in vitro*, as well as of mediator releasing cells *in vitro*. It is not known whether such factores are a primary abnormality in BHR.

BHR is a major pathophysiological characteristic of obstructive airway disease. The degree of BHR has been shown to correlate with the severity of the disease and with the amount of drug treatment required to control symptoms. Prospective studies are needed to confirm indications that the degree of BHR is related to the prognosis of asthmatic children and the progress of chronic airflow limitation.

References

1. Tiffeneau R, Beauvallet M. Epreuve de bronchoconstriction et de bronchodilatation par aérosols. Emploi pour le dépistage, la mesure et la controle des insuffisances respiratoires chroniques. Bull Acad Natl Med 1945; 129: 165-8.

2. Tiffeneau R. Hypersensibilité cholinergo-histaminique pulmonaire de l'asthmatique. Acta Allergologica 1958, suppl 5:187-221.

3. Curry JJ. The action of histamine on the respiratory tract in normal and asthmatic subjects. J Clin Invest 1946; 25:785-91.

4. Curry JJ. Comparative action of acetyl-beta-methylcholine and histamine on the respiratory tract in normals, patients with hay fever, and subjects with bronchial asthma. J Clin Invest 1946; 26:430-8.

5. De Vries K, Goei JT, Booij-Noord H, Orie NGM. Changes during 24 hours in the lung function and histamine hyperreactivity of the bronchial tree in asthmatic and bronchitis patients. Int Arch Allergy 1962; 20:93-101.

6. Langen De JE, Booy-Noord H. The influence of histamine and adrenaline on the vital capacity. Int Arch Allergy 1957; 11: 279-84.

7. De Vries K.Histamine thresholds and initial lung function values.In: Bronchitis III Third Int. Symposium. Orie NGM, Lende van der R (eds). Van Gorcum, Assen 1970: 359-62.

8. Vries de K, Booij-Noord H, Goei JT, Grobler NJ, Sluiter HJ, Tammeling GJ, Orie NGM. Hyperreactivity of the bronchial tree to drugs, chemical, and physical agents. In Bronchitis II. Orie NGM, Sluiter HJ (eds). Van Gorcum, Assen. 1964:167-80.

9. Booij-Noord H, Grobler NJ Orie NGM, Vries de K. Protective action of various drugs on provocation tests with respiratory irritants in patients with CNSLD. Respiration 1969; 26: 182-95.

10. Gökemeyer JDM. Hyperreactiviteit van de luchtwegen. Thesis. Groningen, 1976.

11. Vries K de, Gökemeijer JDM, Koëter GH, Monchy JGR de, Bork LE van, Kauffman HF, Meurs H. Cholinergic and adrenergic mechanisms in bronchial hyperreactivity. In: Bronchial hyperreactivity. Morley J (ed.). Academic Press London 1982: 107-21.

12. Boushey HA, Holtzman MJ, Shelter JR, Nadel JA. State of the art: Bronchial hyperreactivity. Am Rev Respir Dis 1980; 121:389-413

13. Simonsson BG, Jacobs FM, Nadel JA. Role of the autonomous nervous system and the cough reflex in the increased responsiveness of airways in patients with obstructive airways disease. J Clin Invest 1967; 46:1812-8.

14. Nadel JA, Salem H, Tamplin B, Tokiwa Y. Mechanisms of bronchoconstriction during inhalation of sulfur dioxide. J Appl Physiol 1965; 20:164-7.

15. Nadel JA, Comroe JH. Acute effects of inhalation of cigarette smoke on airway conduction. J Appl Physiol 1961; 16: 713.

16. DuBois AB, Dautrebande L. Acute effects of breathing inert particles and of carbachol aerosol on the mechanical characteristics of the lung in man. Changes in response after inhaling sympathicomimetic aerosols. J Clin Invest 1958; 37: 1746-55.

17. Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. Clin Allergy 1977; 7: 235-43.

18. Eiser NM, Kerrebijn KF, Quanjer PhH. Guidelines for standardization of bronchial challenge with (nonspecific) bronchoconstricting agents. Working group: Bronchial hyperreactivity SEPCR. Bull Eur Physiopath Resp 1983; 19:495-514.

19. Eiser NM. Bronchial provocation tests. In: Bronchial hyperresponsiveness, normal and abnormal control, assessment and therapy. Nadel JA, Pauwels R, Snashall PD (eds). Blackwell Scientific Publications, Oxford 1987;173-254.

20. Holtzman MJ, Sheller JR, Dimeo M, Nadel JA, Boushey HA. Effect of ganglionic blockade on bronchial reactivity in atopic subjects. Am Rev Respir Dis 1980; 122:17-25.

21. Cartier A, Malo JL, Begin P, Sestier M, Martin RR. Time course of the bronchoconstriction induced by inhaled histamine and methacholine. J Appl Physiol 1983; 54:821-6.

22. Kaliner M, Orange RP, Austen KF. Immunological release of histamine and slow reacting substance of anaphylaxis from human lung. IV: Enhancement by cholinergic and alpha-adrenergic stimulation. J Exp Med 1972; 136:556-67.

23. McNeil RS. Effect of beta-adrenergic blocking agent propanolol on asthmatics. Lancet 1964; 2:1101-2.

24. Richardson PS, Sterling GM. Effects of beta-adrenergic receptor blockade on airway conductance and lung volume in normal and asthmatic subjects. Br Med J 1969; 3:143-5.

25. Foresi A, Chetta A, Corbo GM, Cuomo A, Olieveri D. Provocative dose and dose-response curve to inhaled propranolol in asthmatic patients with bronchial hyperresponsiveness to methacholine. Chest 1987; 92:455-59.

26. Grieco MH, Pierson RN. Mechanism of bronchoconstriction due to beta-adrenergic blockade. J Allergy Clin Immunol 1971; 48:143-52.

27. Barnes PJ. State of the art: Neural control of human airways in health and disease. Am Rev Respir Dis 1986; 134:1289-1314.

28. Koëter GH, Meurs H, De Monchy JGR, De Vries K. Protective effect of disodium cromoglycate on propranolol challenge. Allergy 1982; 37:587-90.

29. Koëter GH, Meurs H. The beta-adrenergic system and airway reactivity. Thesis. Groningen, 1984.

30. Sterk PJ, Plomp A, Crobach MJJS, Van de Vate JF, Quanjer PhH. The physical properties of a jet-nebulizer and their relevance for the histamine provocation test. Bull Europ Physiophath Resp 1983; 19:27-36.

31. Cockcroft DW. Measurement of airway responsiveness to inhaled histamine or methacholine: method of continuous aerosol generation and tidal breathing inhalation. In: Airway responsiveness. Hargreave FE, Woolcock AJ (eds). Astra Pharmaceuticals, Mississauga, Canada 1985; 22-8.

32. Gayrard P, Orehek J, Grimaud C, Charpin J. Bronchoconstrictor effects of deep inspiration in patients with asthma. Am Rev Respir Dis 1975; 111:433-9.

33. Pennock BE, Rogers RM, McCaffree DR. Changes in measured spirometric indices. What is significant? Chest 1981; 80:97-9.

34. Tattersfield AE, Keeping IM. Assessing change in airway calibre; measurements of airway resistance. Br J Clin Pharmac 1979; 8:307-19.

35. Ariens EJ. Pharmacology of airway smooth muscle. In: Bronchial hyperresponsiveness, normal and abnormal control, assessment and therapy. Nadel JA, Pauwels R, Snashall PD (eds). Blackwell Scientific Publications, Oxford 1987; 7-22.

36. Brain JD, Valberg PA. Deposition of aerosol in the respiratory tract. Am Rev Respir Dis 1979; 120:1325-73.

37. Heyder J. Mechanisms of aerosol particle deposition. Chest 1981; 80:820-3.

38. Dolovich, MB, Sanchis J, Rossman C, Newhouse MT. Aerosol penetrance: a sensitive index of peripheral airways obstruction. J Appl Physiol 1976; 40: 468-71.

39. Hogg JC. Bronchial mucosal permeability and its relationship to airway hyperreactivity. J Allergy Clin Immunol 1981; 67:421-5.

40. Hahn HL. Role of the parasympathetic nervous system and of cholinergic mechanisms in bronchial hyperreactivity. In: Cellular basis of bronchial hyperresponsiveness. Kerrebijn KF, Eiser NM (eds). Bull Eur Pathophysiol Respir 1986; 22 (suppl 7): 112-42.

41. Fuller RW, Dixon CMS, Dollery CT, Barnes PJ. Inflammatory mediator interaction in asthma: prostaglandin D_2 potentiation of histamine and cholinergic hyperresponsiveness. Am Rev Respir Dis 1986; 133:252-4.

42. Moreno RH, Hogg JC, Pare PD. Mechanics of airway narrowing. Am Rev Respir Dis 1986; 133:1171-80.

43. Freedman BJ. The functional geometry of the bronchi. Bull Physiophathol Respir 1972; 8:545-51.

44. Woolcock AJ, Salome CM, Yan K. The shape of the dose-response curve to histamine in asthmatic and normal subjects. Am Rev Respir Dis 1984; 130:71-5.

45. Sterk PJ, Daniel EE, Zamel N, Hargreave FE. Limited bronchoconstriction to methacholine using partial flow-volume curves in non-asthmatic subjects. Am Rev Respir Dis 1985; 132:272-7.

46. Neyens HJ. Bronchial responsiveness in children. Thesis. Rotterdam; 1981.

47. Cockcroft DW, Berscheid BA, Murdock KY. Measurement of responsiveness to inhaled histamine using FEV_1: comparison of PC_{20} and threshold. Thorax 1983; 38:523-7.

48. Kalsner S. A new approach to the measurement and classification of forms of supersensitivy of autonomic effector responses. Br J Pharmacol 1974; 51: 427-34.

49. Thomson NC, Roberts RS. Measurement of effect of drugs on airway responsiveness. In: Airway responsiveness. Hargreave FE, Woolcock AJ (eds.). Astra Pharmaceuticals, Mississauga, Canada 1985; 105-118.

50. Snashall PD. Mechanisms of hyperresponsiveness, general review. In: Bronchial hyperresponsiveness, normal and abnormal control, assessment and therapy. Nadel JA, Pauwels R, Snashall PD (eds.). Blackwell Scientific Publications Oxford 1987; 257-314.

51. Hargreave FE, Dolovich J, O'Byrne PM, Ramsdale EH, Daniel EE. The origin of airway hyperresponsiveness. J Allergy Clin Immunol 1986; 78:825-32.

52. Vries de K. Clinical significance of bronchial hyperresponsiveness. In: Bronchial hyperresponsiveness, normal and abnormal control, assessment and therapy. Nadel JA, Pauwels R, Snashall PD (eds.). Blackwell Scientific Publications Oxford 1987; 359-71.

53. Orie NGM, Sluiter HJ, Vries K de, Tammeling GJ, Witkop J. The host factor in bronchitis. In: Bronchitis; an international symposium. Groningen 1960; 43-59.

54. Pauwels R. Heriditary factors in airway responsiveness. In: Bronchial hyperresponsiveness, normal and abnormal control, assessment and therapy. Nadel JA, Pauwels R, Snashall PD, eds. Blackwell Scientific Publications, Oxford 1987; 337-41.

55. Golden JA, Nadel JA, Boushey HA. Bronchial hyperreactivity in healthy subjects after exposure to ozone. Am Rev Respir Dis 1978; 118:287-94.

56. Empey DW, Laitinen LA, Jacobs L, Gold WM, Nadel J. Mechanisms of bronchial hyperreactivity in normal subjects after upper respiratory tract infections. Am Rev Respir Dis 1976; 113:131-9.

57. Juniper EF, Frith PA, Hargreave FE. Long-term stability of bronchial responsiveness to histamine. Thorax 1982; 37:288-91.

58. Platts-Mills TAE, Torey ER, Mitchell EB, Mozarro H, Nock P, Wilkins SR. Reversal of bronchial hyperreactivity during prolonged allergen avoidance. Lancet 1982; 2:675-8.

59. Chan.Yeung M, Lam S, Tse KS. Measurement of airway responsiveness in occupationa; asthma. In: Airway responsiveness. Hargreave FE, Woolcock AJ (eds.). Astra Pharmaceuticals, Mississauga, Canada 1985; 129-34.

60. Löwhagen O. Drug influences on bronchial responsiveness. In: Bronchial hyperresponsiveness, normal and abnormal control, assessment and therapy. Nadel JA, Pauwels R, Snashall PD, eds. Blackwell Scientific Publications, Oxford 1987; 385-408.

61. Ryan G, Latimer KM, Dolovich J, Hargreave FE. Bronchial responsiveness to histamine: relationship to diurnal variation of peak flow rate, improvement after bronchodilator, and airway caliber. Thorax 1982; 37:423-9.

62. Ramsdale EH, Roberts RS Morris MM, Hargreave FE. Differences in responsiveness to hyperventilation and methacholine in asthma and chronic bronchitis. Thorax 1985; 40:422-6.

63. Yan K, Salome CM, Woolcock AJ. Prevalence and nature of bronchial hyperresponsiveness in subjects with chronic obstructive pulmonary disease. Am Rev Respir Dis 1985; 132:25-9.

64. Tattersfield AE. Relationship of resting airway calibre to measurements of airway responsiveness in man. In: Airway responsiveness. Hargreave FE, Woolcock AJ (eds.). Astra Pharmaceuticals, Mississauga, Canada 1985; 94-102.

65. Dunnill MS. The pathology of asthma, with special reference to change in the bronchial mucosa. J Clin Path 1960; 13:27-33.

66. Tattersfield AE.Measurement of bronchial ractivity; a question of interpretation. Thorax 1981; 36: 561-5.

67. Barnes PJ. The third nervous system in the lung: physiology and clinical perspectives. Thorax 1984; 39:561-7.

68. Richardson JB. Nerve supply to the lungs. Am Rev Respir Dis 1979; 119:785-802.

69. Daniel EE, Davis C, Jones T, Kannan MS. Control of airway smooth muscle. In: Airway rectivity. mechanisms and clinical relevance. Astra, Ontario 1980:80-107.

70. Boushey HA. Bronchial challenge by physical agents. Clin Rev Allergy 1985; 3:411-26.

71. Boushey HA, Holtzman MJ. Autonomic regulation of airways: parasympathic nervous system. In: Bronchial asthma, mechanisms and theraeutic. Weis EB, Segal MS, Stein M (eds.). Little Brown, Boston 1985; 2nd edition:111-22.

72. Postma DS, Keijzer JJ, Koëter GH, Sluiter HJ, De Vries K. Influence of the parasympathetic and sympathetic nervous systems on nocturnal bronchial obstruction. Clin Sci 1985; 69: 251-8.

73. Barnes PJ. Muscarinic receptor subtypes: implications for lung disease. Thorax 1989; 44: 161-7.

74. Laitinen LA, Heino M, Laitinen A, Kava T, Haahtela T. Damage of the airway epithelium and bronchial reactivity in patients with asthma. Am Rev Respir Dis 1985; 131:599-606.

75. Holtzman MJ. Lipoxygenation of arachidonic acid in tracheal epithelial cells. In: Cellular basis of bronchial hyperresponsiveness. Kerrebijn KF, Eiser NM (eds.). Bull Eur Pathophsiol Respir 1986; 22 (suppl 7):38.

76. Cuss FM, Barnes PJ. Epithelial mediators. Am Rev Respir Dis 1987; 136, suppl Airway smooth muscle and disease: S25-35.

77. Jongste JC de, Mons H, Bonta IL, Kerrebijn KF. Cholinergic and non-adrenergic inhibitory nerve-mediated responses of isolated human airways from patents with and without COPD or asthma.J Appl Physiol 1988.

78. Laitinen A, Partanen M, Hervonen A, Laitinen LA. Electron microscopic study on the innervation of human lower respiratory tract. Eur J Respir Dis 1985; 67:209-15.

79. Richardson JB, Ferguson CC. Neuromuscular structure and function in the airways. Fed Proc 1979; 38:202-8.

80. Cabezas GA, Graf PD, Nadel JA. Sympathetic versus parasympathetic nervous regulation of airways in dogs. J Appl Physiol 1971; 31:651-5.

81. Skoogh BE. Airway ganglia. Bull Eur Physiopath Respir 1986; 22 (suppl 7):143-7.

82. Grieco MH, Pierson RN. Mechanism of bronchoconstriction due to beta-adrenergic blockade. J Allergy Clin Immunol 1971; 48:143-52.

83. Barnes PJ, Brown MJ, Silverman M, Dollery CT. Circulating catecholamines in exercise and hyperventilation-induced asthma. Thorax 1981; 36:435-40.

84. Warren JB, Keynes RJ, Brown MJ, Jenner DA, McNicol NW. Blunted sympatho-adrenal response to exercise in asthmatic subjects. Br J Dis Chest 1982; 76:147-50.

85. Ind PW, Brown MJ, Barnes PJ. Sympathoadrenal responses in asthma. Thorax 1983; 38:702.

86. Inoue S. Effects of epinephrine on asthmatic children. J Allergy 1967; 40:337-48.

87. Middleton E, Finke SR. Metabolic response to epinephrine in bronchial asthma. J Allergy 1968; 42:288-99.

88. Parker CW, Smith JW. Alterations in cyclic AMP metabolism in human bronchial asthma. I. Leukocyte responsiveness to beta-adrenergic agents. J Clin Invest 1973; 52:48-59.

89. Patel KR, Alston WC, Kerr JW. The relationship of leukocyte adenylate cyclase activity and airways response to beta-blockade and allergen challenge in extrinsic asthma. Clin Allergy 1974; 4:311-22.

90. Szentivanyi A. The beta-adrenergic theory of the atopic abnormality in bronchial asthma. J Allergy 1968; 42:203-32.

91. Busse W. Decreased granulocyte response to isoproterenol in asthma during upper respiratory tract infections. Am Rev Respir Dis 1977; 115:783-90.

92. Meurs H, Koëter GH, De Vries K, Kauffman HF. The beta-adrenergic system and allergic bronchial asthma: changes in lymphocyte beta-adrenergic number and adenylate cyclase activity after an allergen-induced asthmatic attack. J Allergy Clin Immunol 1982; 70:272-80.

93. Koëter GH, Meurs H, Kauffman H, Monchy JGR de, Sluiter HJ, Vries K de. Changes in the beta-adrenergic system in bronchial asthma induced by terbutaline. Agents and actions 1983; 13:259-64.

94. Meurs H, Kauffman H, Koëter GH, Timmermans A, Vries K de. Regulation of the beta-receptor-adenylate cyclase system in lymphocytes of allergic patients with asthma: Possible role for protein kinase C in allergen-induced nonspecific refractoriness of adenylate cyclase. J Allergy Clin Immunol 1987; 80:326-39.

95. Bruynzeel PLB, Berg W van den, Hamelink ML, Bogaard W van den, Houbel LAM, Kreukniet J. Desensitization of the beta-adrenergic receptor on leukocytes after long-term oral use of a beta-sympathicomimetic;its effect on the beta-adrenergic blockade hypothesis of Szentivanyi. Ann Allergy 1979; 43:105-9.

96. Avner BP, Jenne JW. Desensitization of isolated bronchial smooth muscle to beta-receptor agonists. J Allergy Clin Immunol 1981; 68:51-7.

97. Harvey JE, Tattersfield AE. Airway response to salbutamol: effect of regular salbutamol inhalations in normal, atopic, and asthmatic subjects. Thorax 1982; 37:280-7.

98. Tashkin DP, Conolly ME, Deutsch RJ, Hui KK, Littner M, Scarpace P, Abrass J. Subsensitization of beta-adrenoreceptors in airways and lymphocytes of healthy and asthmatic subjects. Am Rev Respir Dis 1982; 125: 185-93.

99. Jenne JW, Chick TW, Strickland RD, Wall FJ. Subsensitivity of beta responses during therapy with a long-acting beta-2 preparation. J Allergy Clin Immunol 1977; 59:383-90.

100. Plummer AL. The development of drug tolerance to beta-2-adrenergic agents. Chest 1978; 73 (suppl) :949-56.

101. Peel ET, Gibson GJ. Effect of long-term inhaled salbutamol therapy on the provocation of asthma by histamine. Am Rev Respir Dis 1980; 121:973-8.

102. Jenne JW. Whither beta-adrenergic tachyphylaxis. J Allergy Clin Immunol 1982; 70:413-6.

103. Uddman R, Sundler F. Neuropeptides in the airways: a review. Am Rev Respir Dis 1987; 136, suppl: The role of neuropeptides in regulating airway function:S3-S8.

104. Richardson J, Beland J. Nonadrenergic inhibitory nervous system in human airways. J Appl Physiol 1976; 41:764-71.

105. Diamond L, O'Donnell M. A non-adrenergic vagal inhibitory pathway to feline airways. Science 1980; 208:185-8.

106. Palmer JBD, Cuss FMC, Barnes PJ. VIP and PHM and their role in non-adrenergic inhibitory responses in isolated human airways. J Appl Physiol 1986; 61:1322-8.

107. Carstairs JR, Barnes PJ. Visualization of vasoactive intestinal peptide receptors in human and guinea pig lung. Pharmacol Exp Ther 1986; 239:249-55.

108. Lundberg JM, Saria A, Brodin E, Rosell S, Folkers R. A substance P antagonist inhibits vagally induced increase in vascular permeability and bronchial smooth muscle contraction in the guinea-pig. Proc Nat Acad Sci USA 1983; 80:1120-4.

109. Lundberg JM, Martling CR, Saria A. Substance P and capsaicin-induced contraction of human bronchi. Acta Physiol Scand 1983; 119:49-53.

110. Lundsberg JM. Lundblad L, Martling CR, Saria A, Stjarne P, Anngard A. Coexistence of multiple peptides and classic transmitters in airway neurons. Am Rev Respir Dis 1987; 136, suppl. The role of neuropeptides in regulating airway function: S16-S22.

111. Fuller RW, Maxwell DL, Dixon CMS, et al. The effects of substance P on cardiovascular and respiratory function in human subjects. J Appl Physiol 1987; 62:1473-9.

112. Barnes PJ. Neuropeptides in human airways. Am Rev Respir Dis 1987; 136, suppl. The role of neuropeptides in regulating airway function:S77-S83.

113. Barnes PJ. Asthma as an axon reflex. Lancet 1986; 2:242-5.

114. Nadel JA. Inflammation and asthma. J Allergy Clin Immunol 1984; 73:651-3.

115. Chung KF. Role of inflammation in the hyperreactivity of the airways in asthma. Thorax 1986; 41:657-62.

116. O'Byrne PM, Hargreave FE, Kirby JG. Airway inflammation and hyperresponsiveness. Am Rev Respir Dis 1987; 136:S35-7.

117. Orehek J, Massari JP, Gayrard P, Grimaud C, Charpin J. Effect of short-term low-level nitrogen dioxide exposure on bronchial sensitivity in asthmatic patients. J Clin Invest 1976; 57:301-7.

118. Cartier A, Thomson NC, Frith PS, Roberts R, Hargreave FE. Allergen-induced increase in bronchial responsiveness to histamine: Relationship to the late asthmatic response. J Allergy Clin Immunol 1982; 70:170-7.

119. Mapp C, Boschetto D, Vechio LD, Crescioli S, Marzo de N, Parleari D, Fabbri LM. Protective effect of anti-asthmatic drugs on late asthmatic reactions and increased airway responsiveness induced by toluene diisocyanate in sensitized subjects. Am Rev Respir Dis 1987; 136:1403-7

120. Lam S, Tan F, Chan H, Chan-Yeung M. Relationship between types of asthmatic reactions, non-specific bronchial reactivity and IgE antibodies in patients with red cedar asthma. J Allergy Clin Immunol 1983; 72:134-139

121. Booij-Noord H, Orie NGM, De Vries K. Immediate and late bronchial obstructive reactions to inhalation of house dust and protective effects of disodium cromoglycate and prednisolone. J Allergy Clin Immunol 1971; 48:344-54.

122. Curzen N, Rafferty P, Holgate ST. Effects of a cyclo-oxygenase inhibitor flurbiprofen, and an H_1 histamine-receptor antagonist terfenadine alone and in combination on allergen-induced immediate bronchoconstriction in man. Thorax 1987; 42:946-52.

123. Booij-Noord H, Quanjer PhH, Vries de K. Protektive Wirkung von Berotec bei Provokationstesten mit spezifischer Allergen Inhalation und Histamin. Int J Clin Pharmacol 1972; 4:69-72.

124. Star van der JG, Berg WChr, Steenhuis EJ, Vries de K. Invloed van beclomethason-dipropionaat per aerosol op de obstructieve reactie in de bronchiën na huisstofinhalatie. Ned T Geneesk 1976; 120:1928-32.

125. Booij-Noord H, De Vries K, Sluiter HJ, Orie NGM. Late bronchial obstructive reaction to experimental inhalation of house dust extract. Clin Allergy 1972; 2:43-61.

126. De Monchy JGR, Kauffman HF, Venge P, Koëter GH, Jansen HM, Sluiter HJ, De Vries K. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. Am Rev Respir Dis 1985; 131:373-6.

127. Richerson HB, Metzger WJ, Hunninghake GW. Experimental models of bronchial asthma in man and the rabbit. In: Asthma: Clinical pharmacology and therapeutic progress.Kay AB (ed.)Blackwell Scientific Publications; 1986.

128. Larsen GL.Animal models of the late asthmatic response.In: The allergic basis of asthma.Kay AB (ed.).Clin Immunol and Allergy 1988; 2:91-110.

129. Kay AB, Austen FR. The IgE mediated release of an eosinophil leukocyte chemotactic factor from human lung. J Immunol 1971; 107:899-902.

130. Nagy L,Lee TH, Kay AB. Neutrophil chemotactic activity in antigen-induced late asthmatic reactions. N Engl J Med 1982; 306:497-501.

131. Robinson C,Holgate ST.Mast cell-dependent inflammatory mediators and their putative role in bronchial asthma.Clin Sci 1985; 68:103-12.

132. Page CP. The role of platelet activating factor in asthma J Allergy Clin Immunol 1988; 81: 144-50.

133. Durham SR, Carroll M, Walsh GM, Kay AB. Leukocyte activation in allergen-induced late-phase asthmatic reactions. N Engl J Med 1984; 311:1398-402.

134. Moqbel R, Durham SR, Shaw RJ, Walsh GM, MacDonald AJ, Mackay JA, Carroll MP, Kay AB. Enhancement of leucocyte cytotoxicity after exercise-induced asthma. Am Rev Respir Dis 1986; 133:609-13.

135. Frigas E, Loegering DA, Solley GO, Farrow GM, Gleich GJ. Elevated levels of the eosinophil granule major basic protein in sputum of patients with bronchial asthma. Mayo Clin Proc 1981; 56:345-53.

136. Holtzman MJ, Fabbri LM, O'Byrne PM, et al. Importance of airway inflammation for hyperresponsiveness induced by ozone. Am Rev Respir Dis 1983; 127:686-90.

137. Boschetto P, Fabbri LM, Zocca E, Milani SG, Pivirotto F, Vechio dal A, Plebani M, Mapp CE. Prednisone inhibits late asthmatic rections and airway inflammation induced by toluene diisocyanate in sensitized subjects. J Allergy Clin Immunol 1987; 80:261-9

138. Lam S, Le Riche J, Phillips D, Chan-Yeung M. Cellular and protein changes in bronchial lavage fluid after late asthmatic reaction in patients with red cedar asthma. J Allergy Clin Immunol 1987; 80:44-50.

139. Cockcroft DW. Mechanism of perennial allergic asthma. Lancet 1983; 2:253-6.

140. Murphy KR, Wilson MC, Irvin CG,Larsen GL et al. The requirement for polymorphonuclear leukocytes in the asthmatic response and heightened airway reactivity in an animal model. Am Rev Respir Dis 1986; 134:62-8.

141. Murlas CG, Roum JH. Sequence of pathologic changes in the airway mucosa of guinea pigs during ozone-induced bronchial hyperreactivity. Am Rev Respir Dis 1985; 131:314-20.

142. Durham SR, Granek BJ, Hawkins R, Newman Taylor AJ. The temporal relationship between increases in airway responsiveness to histamine and late asthmatic responses induced by occupational agents. J Alergy Clin Immunol 1987; 79:398-406.

143. O'Byrne PM, Leikauf GD, Aizawa H, Bethel RA, Ueki IF, Holtzman MJ, Nadel JA. Leukotriene B_4 induces airway hyperresonsiveness in dogs. J Appl Physiol 1985; 59: 1941-1946.

144. Aizawa H, Chung KF, Leikauf GD.et al.. Significance of tromboxane generation in ozone-induced airway hyperresponsiveness in dogs. J Appl Physiol 1985; 59:1918-23.

145. Cuss FM, Dixon CMS, Barnes PJ. Effects of inhaled platelet activating factor on pulmonary function and bronchial responsiveness in man. Lancet 1986;2:189-92.

146. Chung KF, Aizawa H, Becker AB, Frick O, Gold WM, Nadel JA. Inhibition of antigen-induced airway hyperresponsiveness by a tromboxane-synthetase inhibitor (OKY046) in allergic dogs. Am Rev Respir Dis 1986; 134: 258-61.

147. Persson CGA. Leakage of macromolecules from the tracheobronchial microcirculation. Am Rev Respir Dis 1987; 135: S71-5.

148. Jansen HM, Out TA. Bronchovascular and mucosal permeability in BHR. In: New developments in mechanisms and treatment of bronchial obstruction. Symposium Astra, Santiago de Compostela, 1987:31-8.

149. Barnes PJ, Cuss FM, Palmer PB. The effect of airway epithelium on smooth muscle contractibility in bovine trachea. Br J Pharmacol 1985; 86:685-91.

150. Kerrebijn KF. Triggers of airway inflammation. Eur J Respir Dis 1986; 69:98-104.

151. Pauwels R. Effect of inflammation on bronchial responsiveness. In: Bronchial hyperresponsiveness, normal and abnormal control, assessment and therapy. Nadel JA, Pauwels R, Snashall PD (eds.). Blackwell Scientific Publications Oxford 1987; 315-21.

152. Vincenc KS, Black JL, Yan K, Armour CL,Donnelly PD, Woolcock AJ. Comparison of *in vivo* and *in vitro* responses to histamine in human airways. Am Rev Respir Dis 1983; 128: 875-9.

153. Armour CL, Lazar NM, Schellenberg RR, Taylor SM, Chan N, Hogg JC, Pare PD. A comparison of *in vivo* and *in vitro* human airway reacivity to histamine. Am Rev Respir Dis 1984; 129: 907-10.

154. Cerrina J, Le Roy Ladurie M, Labat C,Raffestin B, Bayol A, Brink C. Comparison of human bronchial muscle respones to histamine and isoproterenol agonists *in vitro*. Am Rev Respir Dis 1986; 134:57-61.

155. Jongste de JC, Mons H, Block R, Bonta IL, Frederiksz AP, Kerrebijn KF. Increased *in vitro* histamine responses in human small airways smooth muscle from patients with chronic obstructive pulmonary disease. Am Rev Respir Dis 1987; 135:549-53.

156. Schellenberg RR, Duff MJ, Foster A, Pare PD. Asthmatic bronchial reactivity *in vitro*. Proc Can Soc Clin Invest 1985; A 202.

157. Jongste de JC. Human airway smooth muscle. Thesis Rotterdam 1987.

158. Antonissen LA, Mitchell RW, Kroeger EA, Kepron W, Tse KS, Stephens NL. Mechanical alterations in a canine asthmatic model. J Appl Physiol 1979; 46:681-7.

159. Neijens HJ, Degenhart HJ, Raatgeep R, Kerrebijn KF. The correlation between increased reactivity of the bronchi and of the mediator releasing cells in asthma. Clin Allergy 1980; 10: 535-9.

160. Neijens HJ, Raatgeep R, Degenhart HJ, Kerrebijn KF. Release of histamine from leukocytes and its determinants *in vitro* in relation to bronchial responsiveness to inhaled histamine and exercise *in vivo*. Clin Allergy 1982; 12:577-86.

161. Neijens HJ, Raatgeep R, Degenhart HJ, Duiverman EJ, Kerrebijn KF. Altered leukocyte responsiveness in relation to the basic abnormality in children with asthma and bronchial hyperresponsiveness. Am Rev Respir Dis 1984; 130:744-7.

162. Taniguchi N, Mita H, Saito H, Yui Y, Kajita T, Shida T. Increased generation of leukotriene C_4 from eosinophils in asthmatic patients. Allergy 1985; 40:571-3.

163. Macklem PT. Bronchial *hypo*responsiveness. Chest 1985: 87S:158S-159S.

164. Sterk PJ, Daniel EE, Zamel N, Hargreave FE. Limited maximal airway narrowing in non-asthmatic subjects. Role of neural control and prostaglandin release. Am Rev Respir Dis 1985; 132:865-70.

165. Parker CD, Bilbo RE, Reed CE. Methacholine aerosol as test for bronchial asthma. Arch Intern Med 1965;115: 452-8.

166. Townley RG, Bewtra AK, Nair NM, Brodkey FD, Watt GD, Burke KM. Methacholine inhalation challenge studies. J Allergy Clin Immunol 1979; 64: 569-74.

167. Cockcroft DW, Berscheid BA, Murdock KY. Unimodal distribution of bronchial responsiveness to inhaled histamine in a random human population. Chest 1983; 83:751-4.

168. Lende van der R, Visser BF, Wever-Hess J, Vries de K, Orie NGM. Distribution of histamine threshold values in a random population. Rev Inst Hyg Mines 1973; 28:186-90.

169. Makino S. Clinical significance of bronchial sensitivity to acetylcholine and histamine in bronchial asthma. J Allergy 1966; 38:127-41.

170. O'Byrne PM, Ryan G, Morris M,Hargreave FE et al. Asthma induced by cold air and its relation to non-specific responsiveness to methacholine. Am Rev Respir Dis 1982; 125:281-5.

171. Weiss JW, Rossing TH, McFadden ER, Ingram RH. Relationship between bronchial responsiveness to hyperventilation with cold air and methacholine in asthma. J Allergy Clin Immunol 1983; 72:140-4.

172. Cockcroft DW, Ruffin RE, Frith PA, Hargreave FE et al. Determinants of allergen-induced asthma: dose of allergens circulating IgE antibody concentration and bronchial responsiveness to inhaled histamine. Am Rev Respir Dis 1979; 120:1053-8.

173. Juniper EF, Frith PA, Hargreave FE. Airway responsiveness to histamine and methacholine: relationship to minimum treatment to control symptoms of asthma. Thorax 1981; 36:575-9.

174. Britton J, Tattersfield AE. Does measurement of bronchial hyperreactivity help in the clinical diagnosis of asthma. Eur J Respir Dis 1986; 68:233-8.

175. Ramsdale EH, Morris MM, Roberts RS, Tech M, Hargreave FE. Asymptomatic bronchial hyperresponsiveness in rhinitis. J Allergy Clin Immunol 1985; 75: 573-7.

176. Postma DS, De Vries K, Koëter GH, Sluiter HJ. Independent influence of reversibility of airflow obstruction and nonspecific hyperreactivity on the long-term course of lung function in chronic air-flow obstruction. Am Rev Respir Dis 1986; 134:276-80.

177. Taylor RG, Joyce H, Gross E, Holland F, Pride NB. Bronchial reactivity to inhaled histamine and annual rate of decline in FEV_1 in male smokers and ex-smokers. Thorax 1985; 40:9-16.

178. Gerritsen J. Prognosis of childhood asthma. Thesis, Groningen 1989.

179. Postma DS, Koëter GH, Vries K de, Sluiter HJ. Clinical implications of airway hyperreactivity. In: New developments in mechanisms and treatment of bronchial obstruction. Symposium Astra, Santiago de Compostela, 1987:153-74.

180. Hogg JC, Macklem PT, Thurlbeck WM. Site and nature of airways obstruction in chronic obstructive lung disease. New Engl J Med 1968; 278: 1355-60.

181. Pelzer AM, Thomson ML. Effect of age, sex, stature, and smoking habits on human airway conductance. J Appl Physiol 1966; 21: 469-74.

182. Eiser NM, Mills J, McRae KD, Shashall PD, Guz A. Histamine receptors in normal human bronchi. Clin Sci 1980; 58:537-44.

183. White J, Eiser NM, Chowienczijk P. Comparison of partial and maximal expiratory flow volume curves for measurement of response to histamine in small airways. Thorax 1982; 37:231.

184. Hyatt RE, Black LF. The flow-volume curve. A current perspective. Am Rev Respir Dis 1973; 107:191-9.

185. Olive JT, Hyatt RE. Maximal expiratory flow and total respiratory resistance during induced bronchoconstriction in asthmatic subjects. Am Rev Respir Dis 1972; 106:366-72.

186. Dawson SV, Elliott EA. *Wave-speed limitation on expiratory flow - a unifying concept.* J Appl Physiol 1977; 43:498-515

187. Black LF, Offord K, Hyatt RE. Variability in the maximum expiratory flow-volume curve in asymptomatic smokers and in non-smokers. Am Rev Respir Dis 1974; 110:282-92.

188. Green M, Mead J, Turner JM. Variability of flow-volume curves. J Appl Physiol 1974; 37: 67-74.

189. Knudson RJ, Burrows B, Lebowitz MD. The maximal expiratory flow volume curve: its use in the detection of ventilatory abnormalities in a population study. Am Rev Resp Dis 1976; 114: 871-9.

190. Gelb AF, Zamel N. Simplified diagnosis of small airways obstruction. N Eng J Med 1973; 288:395-8.

191. Mead J. Analysis of the configuration of maximum expiratory flow-volume curves. J Appl Physiol 1978; 44:156-65.

CHAPTER 2

INFLUENCE OF DRUGS ON BRONCHIAL HYPERRESPONSIVENESS

Introduction

Drugs that have a protective effect on bronchial hyperresponsivenesss can be classified according to their pharmacologic effect on the target organ. It is, however, necessary to define bronchial hyperresponsiveness more precisely taking into account the stimulus used, because through a certain mechanism, a drug may protect against a stimulus, while it has no such effect against another stimulus.

The following classification can be made:

a. Competitive antagonists: antihistamines and anticholinergics interfere with agonist-receptor interactions without producing a response of their own. The antagonist effect can be undone by excess of an agonist.
b. Non-competitive antagonists: antagonists that do not compete with an agonist at the same site on the receptor. None of the anti-asthmatic drugs has this mode of action.
c. Functional antagonists suppress the effect of a stimulus on the target organ, not by interference with the agonist-receptor complex, but by producing an opposite effect. Beta-adrenergic agonists and xanthine derivatives suppress the bronchoconstrictive effect of a cholinergic agent by producing bronchodilatation. Calcium-antagonists can probably also be considered as functional antagonists.
d. Drugs that influence BHR by indirect mechanisms, such as anti-allergic or anti-inflammatory agents.

In this chapter the methodology of and the problems associated with studies of drug-induced effects on BHR are discussed. This introduction is followed by a short review of the pharmacology and clinical studies of currently used drugs.

Measurement of drug-induced changes in smooth muscle responsiveness to constrictor agents in vitro

The effect of a drug on smooth muscle constrictor responsiveness is analysed by studying cumulative log-dose-response curves of a constrictor like histamine in the presence of increasing concentrations of this drug (fig. 1;[1]). When a competitive antagonist is added, e.g. a histamine antagonist, the dose-response curve is shifted rightward, without a change in the maximal constrictor response. Drugs like theophylline or beta-agonists have the same effect on histamine-induced ASM constriction and are called functional antagonists[2]. Non-competitive antagonism leads to a flattening of the dose-response curve and a decrease in the maximal response.

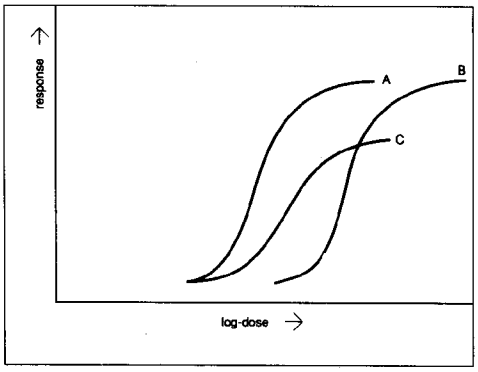

Figure 1. Drug-induced changes in airway smooth muscle responsiveness *in vitro*. A. Pre-treatment curve. B. Decreased responsiveness caused by competitive antagonism, leading to parallel right-ward shift of the curve C. Decreased responsiveness caused by non-competitive antagonism, leading to a decrease in the maximal response.

Measurement of drug-induced changes in bronchial responsiveness to constrictor agents in vivo

Theoretically, drug-induced changes in BR can be analysed *in vivo* in a way analogous to the *in vitro* model. Competitive, functional, and non-competitive antagonists may be expected to induce comparable changes in the dose-response curve *in vivo* and *in vitro*. In addition, for the same geometrical reasons by which inflammatory airway wall oedema amplifies the airway response to a bronchoconstrictor *in vivo*, anti-inflammatory drugs might be expected to decrease the maximal bronchial obstructive response[3,4].

However, the *in vitro* model cannot simply be applied to the *in vivo* situation for two reasons:

- since it is impossible to obtain complete dose-response curves *in vivo* (including the maximal response) in subjects with moderate to severe BHR, the effects of drugs cannot be described in terms of position, slope and maximal response (fig 2;[5]).
- changes in bronchial responsiveness cannot be expected to reflect accurately changes in smooth muscle responsiveness, when baseline airway calibre has been changed simultaneously (see Chapter 1, Mechanisms causing BHR). For mere geometrical reasons, an increase in airway calibre will lead to a decrease in bronchoconstrictor responsiveness[6].

Nevertheless, studying the protective effect of anti-asthmatic drugs on BHR is useful for several reasons:

1. The correlation between airway calibre and BHR is poor, and the degree of BHR varies widely in asthmatic subjects with normal airway calibre[7].
   A broncho-protective effect of anti-asthmatic drugs in these subjects would demonstrate that this effect can be separated from the bronchodilatory effect.
2. The question whether a drug can protect against non-specific stimuli such as cold air, hyperventilation, or chemical agents is at least as relevant as whether the drug has a bronchodilatory effect. In subjects with severe chronic obstructive disease, bronchodilators sometimes have a poor effect on lung function, but a significant protective effect on BHR. This effect is of clinical importance, because it may prevent bronchoconstriction following exposure to non-specific irritants[8,9].
3. The demonstration of effects of certain drugs on BHR may be relevant in the study of the mechanisms of BHR. If, for example, anti-inflammatory lead to an impor-

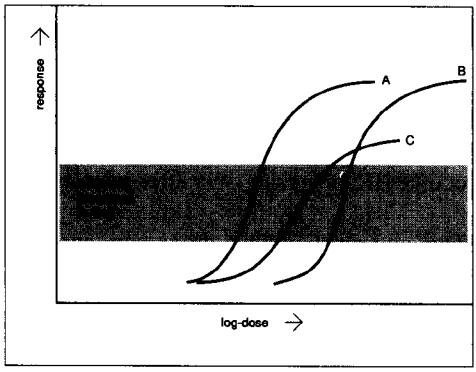

Figure 2. Drug-induced changes in bronchial responsiveness. Curves A, B, and C as in figure 6. With *in vivo* measurement, it is not possible to obtain complete dose-response curves, including threshold and maximal response. Therefore, the nature of the decrease in bronchial responsiveness (deviation (curve B) or non-deviation (curve C)) cannot easily be analysed.

tant improvement in BHR, it may be concluded that inflammatory processes play an equally important role in the mechanisms causing BHR.

To cope with the problem of the mutual influence of changing airway calibre and BR, several study designs have been developed.

A. A comparison of the relationship between changes in airway calibre and BR due to day-to-day variations with the effect of a pharmacological agent on this relationship makes it possible to separate the protective effect of the agent from a change in airway calibre. This approach has been applied by O'Byrne et al.[10]. They compared the effect of a histamine-antagonist on FEV_1 and BR (expressed as histamine PC_{20}) with placebo. Besides a protective effect on the histamine PC_{20}, the drug also had a bronchodilatory effect. Furthermore, the histamine PC_{20} showed a tendency to rise with the FEV_1 on placebo and active drug pretreatment days. From the slope of this relationship between FEV_1 and PC_{20}, the proportion of the PC_{20} change due to the bronchodilatory effect of the agent could be estimated. This amount in PC_{20} change was then subtracted from the total change in PC_{20} after active drug, and thus the "pure" protective effect of the drug was estimated.

B. The protective effect of anti-asthmatic drugs on BHR can be optimally assessed when constrictor dose-response curves are compared that originate from similar base line lung function values. However, because most drugs also improve baseline lung function, it is probably better to study the potency of drugs to protect against BHR in subjects with normal lung function[11]. In studies of this type it is possible to quantify the effect of pharmacological agents on position, slope, and maximal reponse of bronchial response curves, and thus obtaining greater insight in mechanisms causing BHR[12,13].

Definition of the protective effect

The operational definition of the protective effect of a pharmacologic agent on BHR is the change in PC_{20} compared with the baseline value. Since PC_{20} values are log-normally distributed, the change in PC_{20} is preferably expressed either in log-dose change or in doubling doses.

Clinical studies on the influence of anti-asthmatic drugs on bronchial hyperresponsiveness

The effect of drugs on BHR can be immediate, occurring within minutes after administration and lasting for some hours, or sustained after repeated administration of the drug. Alternatively, there may be a long-term effect on BHR, occurring only after maintenance treatment for weeks or possibly months.

In general, the immediate protective effect of bronchodilator drugs can easily be demonstrated. Theoretically, the possibility exists that bronchodilators also have long-term effects. In a study of the long-term effect on BHR, the drug under study is usually withheld for some hours before each inhalation provocation test to preclude the immediate effect.

In the following sections the literature on the effect of drugs is reviewed. If available, data on short-term and long-term effects are presented separately.

Disodium cromoglycate and related drugs

Disodium cromoglycate (DSCG) was introduced about 20 years ago as a drug that appeared to be effective in maintenance treatment of mild allergic asthmatic patients[14]. DSCG has been shown to prevent the early as well as the late asthmatic response to allergen[15]. Besides having an anti-allergic effect this drug has been shown to block the bronchial response to a variety of non-allergic stimuli, such as exercise[16], propranolol[17], and sulphur dioxide inhalation[18]. The drug seems to have several modes of action. DSCG is a potent inhibitor of IgE-dependent mediator release from rat connective tissue mast cells[19]. However, in human lung mast cells this effect appears to be weak[20]. Furthermore the *in vivo* effect of DSCG on mast cell mediator release due to allergen challenge appears to be weaker than the effect of an inhaled beta-agonist[21].

The fact that beta-agonists are not effective in preventing the LAR[22], throws doubt on the importance of the stabilizing effect on the mast cells in the clinical effectiveness of DSCG. It has been demonstrated that late asthmatic reactions and exercise-induced asthmatic reactions are accompanied by activations of blood neutrophils and monocytes, as measured by increased membrane-receptor expression and cytotoxic capacity. DSCG can inhibit this cell activation *in vivo* as well as *in vitro*[23]. This action of DSCG may play an important part in its capacity to block the LAR and the subsequent increase in BR. Another mechanism by which DSCG might inhibit broncho-obstructive reactions to allergic as well as non-allergic stimuli is a blockade of C-fibre afferent activity[24].

Short-term effects Although DSCG inhibits bronchoconstriction induced by exercise[16], propranolol[17], and SO_2 inhalation[18], it does not protect against histamine-induced bronchoconstriction in the acute situation[25].

Long-term effects In addition to inhibiting the EAR and LAR occurring after allergen challenge, DSCG has been shown to prevent the increase in BR that occurs after the allergic reaction[26]. The increase in BR in allergic patients during increased allergen exposure such as in the pollen season can be inhibited by maintenance treatment with DSCG[27].

In a study of Löwhagen, who treated allergic asthmatic patients with perennial symptoms with DSCG for 4 weeks, no significant effect on histamine responsiveness was observed[28]. In another study, in which non-allergic patients were treated for 6 weeks, a slight beneficial effect on BHR was observed[29]. A decrease in cold air hyperresponsiveness has been observed after long-term treatment with DSCG[30].

It may be concluded that maintenance treatment with DSCG can decrease BHR, possibly not exclusively in allergic patients. In general, however, it is only effective in patients with mild symptoms.

Nedocromil

The new compound nedocromil sodium displays many of the known actions of DSCG *in vitro* and has been shown to be at least as effective as DSCG in inhibiting bronchoconstriction induced by allergen, SO_2, fog, and exercise[31]. There is evidence that nedocromil has additional anti-inflammatory effects besides mast-cell stabilization. Like DSCG, it inhibits the increase in BHR in the pollen season in atopic patients[32]. No data are as yet available on the effect of long-term treatment on BHR in (allergic or non-allergic) patients with perennial symptoms.

Glucocorticosteroids

Development of inhalation corticosteroids The beneficial effect of glucocorticosteroids (GCS) in the treatment of asthma has been known since the early fifties[33]. The introduction of GCS with minimalized mineralo-corticosteroid effect, such as prednisolone, was followed by the development of inhaled GCS[34].

The first two GCS that possessed an increased topical as compared with systemic potency were beclomethasone 17a, 21-diproprionate (BDP) and the 16a,17a-acetonide glucocorticosteroid triamcinolone acetonide. Next to their lipophilic side chains, these compounds were halogenated at the 9-position. The weak systemic effect of these GCS is due to the rapid degradation by esterases to compounds with lower activity[35].

The effectiveness of inhaled GCS in maintenance treatment of asthma has now been established.

Budesonide The systemic effects that were noticed after inhalation of high doses of BDP[36], led to the search for GCS with an increased topical vs. systemic effect ratio. It appeared that halogenation at the 6- or 9-position is not necessary to obtain an increased local effect. On the contrary, it leads to an increased systemic effect. For this reason further studies were carried out with non-halogenated GCS. A series of experiments with 16a, 17a acetal glucocorticosteroids, asymmetrically substituted at the 22-position with various side chains, were carried out, and led to the development of budesonide, a 16a,17a acetal GCS with a propyl side chain at the 22-position[37]. It is rapidly biotransformed in inactive compounds in the liver, while such biotransformation does not take place in lung tissue. In animal studies it was demonstrated that the local anti-inflammatory potency of budesonide in the skin was about twice that of BDP, while the systemic effect as measured by the potency to induce thymus involution was three times weaker[37]. Also in human studies the topical potency of budesonide in the skin was about twice that of BDP, while the systemic effect of inhaled budesonide as measured by the depression of plasma cortisol

levels and changes in number of blood lymphocytes, neutrophils, and eosinophils was significantly weaker[38].

Subsequent studies in adults have shown that budesonide can be safely used in maintenance treatment in doses up to 1600 μg/day with only minor systemic side effects[39,40].

Corticosteroids and the late allergic response The inhibitory effect of single doses of GCS on the allergen-induced late asthmatic response has been well-known for many years[15,41,42]. Inhaled GCS have been shown to prevent the early asthmatic reaction after allergen inhalation, when given daily for at least 1 week[43,44]. A single dose of inhaled BDP has also been shown to prevent the allergen-induced increase in BR[26]. Recently it was demonstrated that BDP (1 mg twice daily during 1 week) can prevent the LAR and the subsequent increase in BR which develops after inhalation of toluene diisocyanate[45].

Corticosteroids; mode of action The mode of action of GCS in asthma and BHR is not exactly known.

There is probably not a single mode of action of GCS which makes these drugs effective in the treatment of asthma, but a number of actions operating on different cell types and on different cellular processes[46]. Some of these actions will be briefly summarized.

a. Corticosteroid effects on mast cells and basophils
Histamine release by immunologically stimulated mouse mast cells[47] and human basophils[48] is inhibited when preincubated with GCS. There is, however, no evidence that pharmalogical doses of GCS have any effect on degranulation of isolated human mast cells[49]. It has been demonstrated that allergen-induced release of histamine and arachidonic acid metabolites from human lung fragments is inhibited[49]. Pipcorn et al. showed that pretreatment with topical steroids for 1 week could prevent the release of mast cell mediators during the early response to allergen in rhinitis[50]. It may be that this effect of prolonged treatment is due to depletion of mast cells from the mucosa.

b. Corticosteroid effects on neutrophils and eosinophils
GCS prevent neutrophil and eosinophil chemotaxis and accumulation at sites of inflammation[51]. Furthermore, it has been shown that increased neutrophil IgG (Fc)-receptor and complement-receptor expression, such as can be caused by chemotactic factors, is inhibited by GCS[52]. Release of prostaglandins and leukotrienes by neutrophils can be blocked by GCS[53]. Eosinophil degranulation and Fc-receptor expression have been shown to be inhibited by GCS[54].

c. Corticosteroid effects on monocytes, macrophages and lymphocytes
The number of monocytes and lymphocytes in the peripheral blood and the migration of these cells to inflammatory sites is reduced under influence of GCS[55,56]. GCS inhibit the release of prostaglandins, leukotrienes, and platelet activating factor by monocytes[57]. The production of lymphokines by macrophages, monocytes, and lymphocytes is reduced, which is probably related to the decrease in number of activated T-helper lymphocytes during GCS treatment[58,59]. The importance of lymphokines for the activation of mast cells, neutrophils, and eosinophils has recently been noted[60].

d. Other corticosteroid effects in asthma
Other actions that may be important in the effectiveness of GCS in the treatment of asthma are the effects on the number of beta-adrenergic receptors of inflammatory cells[61] and possibly of ASM cells, an inhibitory effect on vascular permeability caused by mediators[62], and a decrease in mucus production[63].

e. Corticosteroid effects at the intracellular level
At the intracellular level, GCS bind to GCS-receptors after passive diffusion into the cytoplasm. The GCS-receptor complex moves into the cell nucleus and stimulates messenger-RNA formation which results in the production of proteins. The action of these proteins determines the phenotypic response. One of the proteins that have been identified is macrocortin, which is found in macrophages. This protein has been shown to exert an anti-phospholipase-A_2 action, thus inhibiting the cleavage of arachidonic acid from membrane phospholipids, and thereby the release of products of the arachidonic acid metabolism[64]. By this mechanism GCS may interfere with mediator release from inflammatory cells, but also with other processes within the cell membrane, such as receptor expression.

Short-term effects of corticosteroids on bronchial hyperresponsiveness The effect of systemic GCS on BHR was studied for the first time by Tiffeneau who found no change of the BR to acetylcholine after oral cortisone[65]. Two other studies also showed no effect of oral GCS on cholinergic BR, when given up to 5 days before inhalation provocation[66,67].

Long-term effects of corticosteroids on bronchial hyperresponsiveness Oral GCS, given for 4 months, had no effect on histamine BR in a study of Mattoli et al.[68]. In a study in asthmatic children, a beneficial effect of prednisone (60 mg/day, 1 week) was observed on methacholine BR[69]. A beneficial effect on inhaled BDP on histamine BR (400-1200 µg day, periods up to 30 months) was observed in three open studies[70-72]. In a placebo-controlled study, BDP (400 µg daily, 4 weeks) reduced histamine BR, increasing the PC_{20} significantly by about half a doubling dose[73]. In a study of Kerrebijn et al. a significant increase in methacholine BR (1.5 doubling dose) was observed in allergic children after treatment with inhaled budesonide (600 µg per day) during a period up to 6 months[74]. Budesonide effectively inhibits exercise induced-asthma in allergic children when given for 3 weeks in a dosage of 400 µg daily[75].

Beta-adrenergic drugs

Short-time effects on bronchial hyperresponsiveness Beta-adrenergic drugs have a protective effect against a variety of stimuli such as histamine[25,76-78], methacholine[76,77,79,80], eucapnic hyperventilation[81], exercise[82,83], and SO_2 inhalation[84]. The effect on histamine varies from 1 to 4 doubling doses[25,76-78], whereas the effect on methacholine is 3-4 doubling doses[76,77,79]. Inhaled beta-agonists are more effective than oral beta-agonists[77]. The duration of the protective effect is rather short and seems to be shorter than the bronchodilatory effect[80]. The fact that protection can be demonstrated against such a variety of bronchoconstrictive stimuli suggests that the effect is caused by functional antagonism. Beta-agonists bind to specific membrane receptors on the ASM membrane. After this binding, adenylate cyclase is activated, which leads to increased intra-cellular cyclic-

AMP concentration. As a consequence, intra-cellular Ca^{++} levels decrease, which leads to relaxation of the contractile filaments.

Mast cell degranulation may play a role in bronchial obstruction caused by exercise and eucapnic hyperventilation[85]. There is evidence that the protective effect of beta-agonists against these stimuli may partly be mediated by mast cell stabilization[86,87].

Long-term effects on bronchial hyperresponsiveness Little is known about the long-term effects of beta-adrenergic drugs on BHR. Peel and Gibson found that treatment with inhaled salbutamol for 4 weeks had no effect on histamine BR in stable asthmatic patients[88]. Harvey and Tattersfield found no effect of inhaled salbutamol on histamine BR when given for 4 weeks in atopic subjects with or without asthmatic symptoms[89]. In both studies, histamine responsiveness was measured before and after 4 weeks of treatment.

Anticholinergic agents

Short-term effects on bronchial hyperresponsiveness Anticholinergic agents are effective against a number of inhaled stimuli that induce bronchoconstriction, such as fog[84], ozone[90], cigarette smoke[91], carbon dust[92], and citric acid aerosol[93]. While anticholinergic agents protect very effectively against cholinergic agents, they are less effective against histamine-induced bronchoconstriction[94]. In general, the increase in histamine PC_{20} is not more than 1.5 doubling dose while beta-agonists have been reported to shift the histamine dose-response curve up to 4 doubling doses[75-78].

Large inter-individual differences have been observed in the protective effect against bronchoconstriction induced by exercise-, cold air- and hyperventilation[95]. In some patients anticholinergics gave strong protection bronchoconstriction induced by cold air, while in other patients its protection was much weaker, even by high doses[95]. Furthermore, the effect of beta-agonists and DSCG is generally better than that of inhaled anticholinergic agents[96]. These results suggest that the role of cholinergic reflex pathways in bronchoconstriction induced by exercise and hyperventilation may be limited, or varies between patients.

Recently the discovery of various muscarinic receptor subtypes throws a new light on these findings[97]. Besides the M_3 subtype receptor on smooth muscle cells, muscarinic receptors of the M_2 subtype are present on the presynaptic membrane of postganglionic cholinergic fibres[98]. Activation of these receptors inhibits the release of acetylcholine[99]. On the other hand, anticholinergic agents that nonspecifically block both M_2 and M_3 subtype receptors might therefore have a limited bronchodilatory effect.

Long-term effects on bronchial hyperresponsiveness The influence on methacholine BR of treatment with ipratropium bromide inhalations for three weeks was studied by Newcomb et al.[100]. They found an increase in BR measured 24 h after the last ipratropium dose. The effect had disappeared at 48 h. The mechanism behind this is unknown, but an increase in cell-surface muscarinic receptor density, induced by chronic muscarinic blockade, might play a role.

Xanthine derivatives

Theophylline. Effects on bronchial hyperresponsiveness The effect of theophylline on bronchoconstricting stimuli has been studied by several authors. In general, a moderately protective effect has been found against histamine[25,101-105], cholinergic[101,104] and exercise-induced asthma[105]. The maximal effect on histamine and methacholine BR is usually up to 2 doubling doses. The fact that only moderate effects are seen *in vivo*, while the anti-constrictive effect of theophylline *in vitro* is strong, is probably due to the limited dose *in vivo*.

It is well-known that the bronchodilatory effect of theophylline is dose-related, being proportional to the logarithm of the plasma concentration[106]. Theophylline plasma concentrations up to 10-12 $mg.L^{-1}$ already have an important bronchodilatory effect[107].

The dose-effect relation of theophylline on BHR has not been extensively studied. Cockcroft et al. found no protective effect on histamine BR of theophylline plasma concentrations below 10 $mg.L^{-1}$, while plasma concentrations above 10 $mg.L^{-1}$ were effective[25]. In another study no correlation could be found between the protective effect of theophylline on histamine BR and the theophylline plasma concentration[104].

In a recent study, Magnussen et al.[108] reported a dose-related protective effect when histamine challenges at different plasma theophylline levels were carried out within the same patient.

Theophylline. Mode of action The cellular mechanism of action of theophylline is still unclear[109]. Several mechanisms have been proposed. The hypothesis that theophylline causes smooth muscle relaxation by inhibition of phosphodiesterase (PDE) activity which leads to intracellular accumulation of cyclic-AMP is now considered as improbable. It has been demonstrated that theophylline is a poor inhibitor of PDE in therapeutic concentrations[110]. Furthermore, potent PDE inhibitors such as dipyridamole have no bronchodilatory properties[111]. Although theophylline may act partly by increasing the release of adrenal catecholamines, this cannot be considered its main activity[112].

Adenosine, a naturally occurring purine nucleotide that can cause bronchoconstriction in asthmatic subjects can effectively be antagonized by theophylline[113]. It is, however, unknown how important the contribution of adenosine is to bronchoconstriction in asthma. The hypothesis of adenosine antagonism as a mechanism for ASM relaxation of xanthine derivates has become unlikely, after it was demonstrated that the recently developed xanthine enprofylline, which has a bronchodilatory capacity of about 5 times that of theophylline, has no adenosine antagonizing properties[109]. Smooth muscle relaxation by theophylline in therapeutic concentrations may be associated with sequestration of intracellular calcium into mitochondria[114]. At present, it is unknown how important this effect on calcium homeostasis is for the bronchodilatory effect of theophylline. Finally, theophylline has an inhibitory effect on the histamine release by mast cells and basophils[115]. Pauwels et al. demonstrated that theophylline and enprofylline could block the LAR in allergic patients, while there was only a moderate effect on the EAR[116]. These data suggest that theophylline may not exclusively act on ASM.

The fact that theophylline inhibits the ASM constrictive effect of many agonists such as histamine, methacholine, serotonin, prostaglandins, and leukotrienes, supports the view that these effects are caused by functional antagonism[2].

Enprofylline Although theophylline is a potent and widely used bronchodilator it has some important drawbacks. There is a large interindividual variation in theophylline pharmacokinetics due to the variability in the rate of hepatic biotransformation[117]. Theophylline elimination is influenced by age[118], smoking[118], concomitant drug use[119], and disease states such as liver cirrhosis[120]. These variations may lead to plasma concentrations, that are too high or too low.

Next to its anti-asthmatic effects, theophylline has a number of important extrapulmonary effects: central nervous system effects like insomnia, anxiety, and seizures, increased gastric secretion, and increased diuresis. These side effects appear to be related to adenosine antagonism[121]. Adenosine, as a naturally occurring metabolite of purine nucleotides, acts as a local hormone whose regulatory functions involve receptor-mediated processes in many tissues[122]. Other extrapulmonary actions of theophylline like nausea, headache, oesophageal lower sphincter relaxation, and vasodilation are probably not related to adenosine antagonism[121]. Due to the poor predictability of its pharmacokinetics, these dose related side effects of theophylline are frequently seen in clinical practice.

Enprofylline (3-propyl-xanthine) is a recently developed xanthine derivative, with a bronchodilatory potency that is 3 to 5 times higher than that of theophylline[121]. Its actions are not mediated by adenosine antagonism[123], and therefore enprofylline does not share some extrapulmonary actions with theophylline, such as central nervous system effects, increased gastric secretion, and diuretic effects. Other side effects like headache and nausea are seen with equal frequency as in the case of theophylline[124,125].

The pharmacokinetic behaviour of enprofylline is different from theophylline. Enprofylline is hardly metabolized; the unchanged drug is almost completely eliminated by the kidneys by active tubular secretion[126]. In healthy subjects enprofylline plasma half-life is about 1.6h[126]. Furthermore, enprofylline excretion has been shown to be linearly related to the creatinine clearance in patients with impaired renal function[127]. Enprofylline is well absorbed in the gastro-intestinal tract[123]. Due to the short half-life, sustained release formulations are necessary to obtain stable plasma concentrations when the drug is administered orally twice daily. Treatment with enprofyline might be safer than with theophylline because some of the dangerous side effects of theophylline are not seen with enprofylline. Moreover, if the pharmacokinetic behaviour of enprofylline appears to be more predictable, toxic plasma concentrations might be avoided more easily.

Calcium-antagonists

The intracellular free calcium concentration plays a key role in smooth muscle contraction and mast cell degranulation. For this reason the effect of calcium channel blockers like nifedipine and verapamil on bronchial obstruction and BR has been studied extensively[128]. Both drugs appear to protect against exercise induced asthma[129-131], as well as cold air induced bronchoconstriction[132]. Some protection against histamine[133,134] and methacholine[133,135] in asthmatic subjects has been reported by some authors, but could not be confirmed by others[136,137]. Further progress in this field may be expected when calcium channel blockers are developed that can block receptor-operated channels.

Non-steroidal anti-inflammatory drugs

Products of the cyclo-oxygenase pathway of arachidonic acid are potent mediators that have a great number of effects relevant to asthma and BHR. Theoretically non-steroidal anti-inflammatory drugs (NSAID) like aspirin and indomethacin might therefore have beneficial effects in asthma, because they inhibit the release of the cyclo-oxygenase pathway products like prostaglandin $F2_a$, prostaglandin D_2 and thromboxane A_2[138].

In some studies indomethacin has been shown to block the late asthmatic reaction to allergen [139,140]. In another study in humans, the LAR after allergen exposure was not prevented by pretreatment with indomethacin, but the expected increase in BR did not occur[141]. The increase in BR that occurs after ozone inhalation in dogs was prevented by pretreatment with indomethacin[142]. Pretreatment with indomethacin has also been shown to prevent viral-induced BHR in asthmatic patients[143].

In long-term studies in large patient groups, non-steroidal anti-inflammatory drugs had no beneficial effects on symptoms and lung function[144,145]. No studies on the effect of long-term treatment of NSAID's on BHR have been performed. Moreover, the adverse effect of NSAID's in an important subpopulation of CNSLD patients precludes large-scale studies.

References

1. Ariens EJ. Pharmacology of airway smooth muscle. In: Bronchial hyperresponsiveness, normal and abnormal control, assessment and therapy. Nadel JA, Pauwels R, Snashall PD,(eds.). Blackwell Scientific Publications, Oxford 1987; 7-22.
2. Persson CGA. Overview of effects of theophylline. J Allergy Clin Immunol 1986; 78: 780-7.
3. Freedman BJ. The functional geometry of the bronchi. Bull Physiopathol Respir 1972; 8: 545-51.
4. Moreno RH, Hogg JC, Pare PD. Mechanics of airway narrowing. Am Rev Respir Dis 1986; 133:1171-80.
5. Thomson NC, Roberts RS. Measurement of effect of drugs on airway responsiveness. In: Airway responsiveness. Hargreave FE, Woolcock AJ, eds. Astra Pharmaceuticals, Mississauga, Canada 1985; 105-18.
6. Tattersfield AE.Measurement of bronchial ractivity; a question of interpretation. Thorax 1981; 36:561-5.
7. Ryan G, Latimer KM, Dolovich J, Hargreave FE. Bronchial responsiveness to histamine: relationship to diurnal variation of peak flow rate, improvement after bronchodilator, and airway calibre. Thorax 1982; 37:423-9.
8. Higenbottam T, Feyeraband C, Clark TJM. Cigarette smoke inhalation and the acute airway response. Thorax 1980; 35:246-54.
9. Postma DS, Koëter GH, Vries K de, Sluiter HJ. Clinical implications of airway hyperreactivity. In: New developments in mechanisms and treatment of bronchial obstruction. Astra, Santiago de Compostela, 1987; 153-73.
10. O'Byrne PM, Thomson NC, Morris M, Roberts R, Daniel EE, Hargreave FE. The protective effect of inhaled chlorpheniramine and atropine on bronchoconstriction stimulated by airway cooling. Am Rev Respir Dis 1983; 128:611-7.

11. Tattersfield AE. Relationship of resting airway calibre to measurements of airway responsiveness in man. In: Airway responsiveness. Hargreave FE, Woolcock AJ,(eds.). Astra Pharmaceuticals, Mississauga, Canada 1985; 94-103.

12. Bel EH, Veen H van der, Kramps JA, Dijkman JH, Sterk PJ. Maximal airway narrowing to inhaled leukotriene D_4 in normal subjects. Am Rev Respir Dis 1987; 136:979-84.

13. Bel EH, Veen H van der, Dijkman JH, Sterk PJ. Budesonide reduces the maximal degree of airway narrowing to leukotriene D_4 in normal subjects. Am Rev Respir Dis 1989; 139: 427-31.

14. Howell JBL, Altounyan REC. A double blind trial of disodium cromoglycate in the treatment of allergic bronchial asthma. Lancet 1967; 2:539-42.

15. Booij-Noord H, Orie NGM, De Vries K. Immediate and late bronchial obstructive reactions to inhalation of house dust and protective effects of disodium cromoglycate and prednisolone. J Allergy Clin Immunol 1971; 48:344-54.

16. Davies SE. Effect of disodium cromoglycate on exercise-induced asthma. Br Med J 1968; 3:593-4.

17. Koëter GH, Meurs H, De Monchy JGR, De Vries K. Protective effect of disodium cromoglycate on propranolol challenge. Allergy 1982; 37:587-90.

18. Sheppard D, Nadel J, Boushey HA. Inhibition of sulphur dioxide induced by cromoglycate in asthmatic subjects. Am Rev Respir Dis 1981; 124:257-9.

19. Church MK. Cromoglycate-like anti-allergic drugs. A review. Drugs of today 1978; 14: 281-341.

20. Butchers PR, Fullarton JR, Skidmore FF, Thompson LE, Vardey CJ, Wheeldon A. A comparison of the anti-anaphydactic activities of salbutamol and disodium cromoglycate in the rat, the rat mast cell, and in human lung tissue. Br J Pharmacol 1979; 67:23-32.

21. Howarth PH, Durham SR, Lee TH, Kay AB, Church MK, Holgate ST. Influence of albuterol, cromolyn sodium and ipratropium bromide on the airway and circulating mediator responses to allergen bronchial provocation in asthma. Am Rev Respir Dis 1985; 132: 986-92.

22. Booij-Noord H, Quanjer PhH, Vries de K. Protektive Wirkung von Berotec bei Provokationstesten mit spezifischer Allergeninhalation und Histamin. Int J Clin Pharmacol 1972; 4:69-72.

23. Kay AB, Walsh GM, Moqbel R, MacDonald AJ, Nagakura T, Carroll NP, Richerson HB. Disodium cromoglycate inhibits activation of human inflammatory cells *in vitro*. J Allergy Clin Immunol 1987; 80:1-8.

24. Dixon M, Jackson DM,Richards IM. The action of sodium cromoglycate on "C"-fibre endings in the dog lung. Br J Pharmacol 1980; 70:11-3.

25. Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Protective effect of drugs on histamine-induced asthma. Thorax 1977; 32:429-37.

26. Cockcroft DW, Murdock KY. Comparative effects of inhaled salbutamol, sodium cromoglycate, and beclomethasone diproprionate on allergen-induced early asthmatic resposes, late asthmatic responses, and increased bronchial responsiveness to histamine. J Allergy Clin Immunol 1987; 79:734-40.

27. Löwhagen O, Rak S. Modification of bronchial hyperreactivity after treatment with sodium cromoglycate during pollen season. J Allergy Clin Immunol 1985; 75:460-7.

28. Löwhagen O, Rak S. Bronchial hperreactivity after treatment with sodium cromoglycate in atopic asthmatics not exposed to relevant allergens. J Allergy Clin Immunol 1985; 75: 343-7.

29. Rak S, Löwhagen O. The effect of disodium cromoglycate and inhaled budesonide on bronchial hyperreactivity in non-atopic asthma. In: Glucocorticosteroids, inflammation and bronchial hyperreactivity. Hogg JC, Ellul-Micallef R, Brattsand R,(eds.). Exerpta Medica 1985; 99-103.

30. Griffin MP, MacDonald N, McFadden ER. Short- and long-term effects of Cromolyn sodium on the airways rectivity of asthmatics. J Allergy Clin Immunol 1983; 71:331-8.

31. Holgate ST. Nedocromil in asthma. Eur J Respir Dis 1986; 69 (suppl 147): 149-50.

32. Doward AJ, Roberts JA, Thomson NL. Effect of nedocromil sodium on histamine airway responsiveness in gras pollen sensitive asthmatics during the pollen season. Clin Allergy 1986; 16:309-17.

33. Randolph TG, Rollins JP. Effect of cortisone on bronchial asthma. J Allergy 1950; 21: 288-95.

34. Brown HM. The introduction and early development of inhaled steroid treatment. In: Topical steroid treatment for asthma and rhinitis. Mygind N, Clark TJH. Balliere Tindall, Londen, 1980: 66-76.

35. Martin LE, Tanner RJN, Clark THJ, Cochrane GM. Absorption and metabolism of orally administered BDP. Clin Pharmacol Ther 1974; 15:267-75.

36. Toogood JH, Lefcoe NM, Haines DSM, Jennings B, Errington N, et al. A graded dose assessment of the efficacy of beclomethasone diproprionate aerosol for severe chronic asthma. J Allergy Clin Immunol 1977; 59:298-308.

37. Brattsand R, Thalen A, Roempke K, Kallstrom L, Gruvstad E. Development of new glucocorticosteroids with a very high ratio between topical and systemic activities. Eur J Respir Dis 1982; 63 (suppl 122):62-73.

38. Johansson SA, Andersson KE, Brattsand R, Gruvstad E, Hedner P. Topical and systemic glucocorticoid potencies of budesonide and BDP in man. Eur J Clin Pharmacol 1982; 22:523-9.

39. Laursen LC, Taudorf E, Weeke B. High dose inhaled budesonide in treatment of severe steroid-dependent asthma. Eur J Respir Dis 1986; 68:19-28.

40. Toogood JH, Crilly RG, Jones G, Nadeau J, Wells GA. Effect of high dose inhaled budesonide on calcium and phosphate metabolism and the risk of osteoporosis. Am Rev Respir Dis 1988; 138: 57-61.

41. Booij-Noord H, Vries K de, Sluiter HJ, Orie NGM. Late obstructive reactions to experimental inhalation of house dust extract. Clin Allergy 1972; 2:43-61.

42. Pepys J, Davies RJ, Breslin ABV, Hendrick DJ, Hutchcroft B. The effect of inhaled beclomethasone diproprionate and sodium cromoglycate on asthmatic reactions to provocation tests. Clin Allergy 1974; 4:13-24.

43. Van der Star JG, Berg CW, Steenhuis ECJ, De Vries K. Invloed van beclomethason-diproprionaat per aerosol op de obstructieve reactie in de bronchiën na huisstofinhalatie. Ned T Geneeskd 1976; 120:1928-32.

44. Dahl R, Johansson SA. Importance of duration of treatment with inhaled budesonide on the immediate and late bronchial reaction. Eur J Respir Dis 1982; 63 (suppl 122):167-75.

45. Mapp C, Boschetto P, Vecchio LD, Crescioli S, De Marzo N, Palcari D, Fabbri LM. Protective effect of anti-asthma drugs on late asthmatic reactions and increased airway responsiveness induced by toluene diisocyanate in sensitized subjects. Am Rev Respir Dis 1987; 136: 1403-7.

46. Kaliner M. Mechanism of glucocorticosteroid action in bronchial asthma. J Allergy Clin Immunol 1985; 76:321-9.

47. Daeron M, Sterk AR, Hirata F, Ishizaka T. Biochemical analysis of glucocorticoid-induced inhibition of IgE-mediated histamine release from mouse mast cells. J Immunol 1982; 129: 1212-8.

48. Schleimer RP, Lichtenstein LM, Gillespie E: Inhibition of basophil histamine release by anti-inflammatory steroids. Nature 1981; 292:454-5.

49. Schleimer RP, Schulman ES, MacGlashan DW, Peters SP, Hayes EC, Adams GK, Lichtenstein LM, Adkinson NF. Effects of dexamethasone on mediator release from human lung fragments and purified lung mast cells. J Clin Invest 1983; 71:1830-5.

50. Pipkorn U, Proud D, Lichtenstein LM, Kagey-Sobotka A, Norman PS, Naclerio NM. Inhibition of mediator release in allergic rhinitis by pretreatment with topical glucocorticosteroids. N Engl J Med 1987; 316: 1506-10.

51. Clark RAF, Gallin JI, Fauci AS. Effects of *in vivo* prednisone on *in vitro* eosinophil and neutrophil adherence and chemotaxis. Blood 1979; 53:633-41.

52. Gin W, Kay AB. The effect of corticosteroids on monocyte and neutrophil activation in bronchial asthma J Allergy Clin Immunol 1985; 76:675-82.

53. Hirata F, Schiffmann E, Venkatasubramian K, Salomon D, Axelrod J. A phospholipase A_2 inhibitor protein in rabbit neutrophils induced by glucocorticoids. Proc Natl Acad Sci USA. 1980; 77: 2533-6.

54. Frigas E, Gleich GJ. The eosinophil and the pathophysiology of asthma. J Allergy Clin Immunol 1986; 77:527-37.

55. Haynes BF, Fauci AS. The differential effect of *in vivo* hydrocortisone on the kinetics of subpopulations of human peripheral blood thymus-derived lymphocytes. J Clin Invest 1978; 61: 703-7.

56. Dale DC, Fauci AS, Wolff SM, Alternate-day prednisolone: leukocyte kinetics and susceptibility to infection. N Engl J Med 1974; 291:1154-8.

57. Bonney RJ et al Antigen-antibody complexes stimulate the synthesis and release of prostaglandins by mouse peritoneal macrophages. Prostaglandins 1979; 18:605-16.

58. Snyder, DS, Unanue ER. Corticosteroids inhibit murine marcophage Ia expression and IL production. J Immunol 1982; 129:1803-5.

59. Slade JD, Hepburn B. Prednisone-induced alterations of circulating human lymphocyte subsets. J Lab Clin Med 1983; 101:479-87.

60. Richerson HB, Metzger WJ, Hunninghake GW. Experimental models of bronchial asthma in man and the rabbit. In: Asthma, clinical pharmacology and therapeutic progress. Kay AB (ed.). Blackwell, Oxford 1986:23-32.

61. Davies AO, Lefkowitz RJ. Corticosteroids-induced differential regulation of beta-adrenergic receptors in circulating human polymorphonuclear leukocytes and mononuclear leukocytes. J Clin Endocrinol Metab 1980; 51:599-605.

62. Svensjo E, Roempke K. Time-dependent inhibition of bradykinin and histamine-induced increase in microvascular permeability by local glucocorticosteroid treatment. In: Glucocorticosteroids, inflammation and bronchial hyperreactivity. Hogg JC, Ellul-Micallef R, Brattsand R (eds.). Excerpta Medica Amsterdam 1985; 136-144.

63. Marom Z, Shellhamer J, Alling D, Kaliner M. The effects of corticosteroids on mucous glycoprotein secretion from human airways *in vitro*. Am Rev Respir Dis 1984; 129:62-5.

64. Blackwell GJ, Carnuccio E, Di Rosa M, et al.. Macrocortin: a polypeptide causing the antiphospholipase effect of glucocorticoids. Nature 1980; 287:147-9.

65. Tiffeneau R, Dunoyer P. Action de la cortisone sur l'hypersensibilité cholinergique pulmonaire de l'asthmatique. Presse Med 1956; 64:719-21.

66. Wolfe JD, Rosenthal RR, Bleeker E, Laube B, Norman PS, Permutt S. The effect of corticosteroids on cholinergic hyperreactivity. J Allergy Clin Immunol 1979; 63:162.

67. Arkins JA, Schleuter DP, Fink JW. The effect of corticosteroids on methacholine inhalation in symptomatic bronchial asthma. J Allergy 1968; 41:209-16.

68. Mattoli S, Rosati G, Mormile F, Ciappi G. The immediate and short-term effects of corticosteroids on cholinergic hyperreacitivity in asthmatic subjects. Respiration 1984; 46:39.

69. Bhagat RG, Crunstein MM. Effect of corticosteroids on bronchial responsiveness to methacholine in asthmatic children. Am Rev Respir Dis 1981; 131:902-6.

70. Easton JG. Effect of an inhaled corticosteroid on methacholine airway reactivity. J Allergy Clin Immunol 1985; 67:388-90.

71. Clarke PS. The effect of beclomethasone diproprionate on bronchial hyperreactivity. Journal of Asthma 1982; 19:91-3.

72. Juniper EF, Frith PA, Hargreave FE. Long-term stability of bronchial responsiveness to histamine. Thorax 1982; 37:288-91.

73. Ryan G, Latiner KM, Juniper EF, Roberts RS, Hargreave FE. Effect of beclomethasone diproprionate on bronchial responsiveness to histamine in controlled non-steroid dependent asthma. J Allergy Clin Immunol 1985; 75:25-30.

74. Kerrebijn KF, Van Essen-Zandvliet EEM, Neijen HJ. Effect of long-term treatment with inhaled corticosteroids and beta-agonists on the bronchial responsiveness in children with asthma. J Allergy Clin Immunol 1987; 79:653-9.

75. Henriksen JM, Dahl R. Effect of inhaled budesonide alone and in combination with low-dose terbutaline in children with exercise-induced asthma. Am Rev Respir Dis 1983; 128:993-7.

76. Bandouvakis J, Cartier A, Roberts R, Ryan G, Hargreave FE. The effect of ipratropium and fenoterol on methacholine- and histamine-induced bronchoconstriction. Br J Dis Chest 1981; 75:295-305.

77. Salome CM, Schoeffel RE, Woolcock AJ. Effect of aerosol and oral fenoterol on histamine and methacholine challenge in asthmatic subjects. Thorax 1981; 36:580-4.

78. Chung KF, Morgan B, Keyes SJ, Snashall PD. Histamine dose-response relationships in normal and asthmatic subjects. Am Rev Respir Dis 1982; 126:849-54.

79. Chung KF, Snashall PD. Metacholine dose-response curves in normal and asthmatic man: effect of starting conductance and pharmacological antagonism. Clin Sci 1984; 66:665-73.

80. Ahrens RC, Bonham AC, Maxwell GA, Weinbeger MM. A method for comparing the peak intensity and duration of action of aerosolized bronchodilators using bronchoprovocation with methacholine. Am Rev Respir Dis 1984; 129:903-6.

81. O'Byrne PM, Morris M, Roberts R, Hargreave FE. Inhibition of the bronchial response to respiratory heat exchange by increasing doses of terbutaline sulphate. Thorax 1982; 37: 913-7.

82. Godfrey S, König P. Inhibition of exercise-induced asthma by different pharmacological pathways. Thorax 1976; 31:137-43.

83. Neijens HJ, Wesseling T, Kerrebijn KF. Exercise-induced bronchoconstriction as an expression of bronchial hyperreactivity: a study of its mechanism in children. Thorax 1981; 36: 517-22.

84. Booij-Noord H, Grobler NJ, Orie NGM, De Vries K. Protective action of various drugs on provocation tests with respiratory irritants in patients with CNSLD. Respiration 1969; 26: 182-95.

85. Godfrey S. Controversies in the pathogenesis of exercise-induced asthma. Eur J Respir Dis 1986; 68:81-8.

86. Howarth PH, Durham SR, Lee TH, Kay AB, Church MK, Holgate ST. Influence of albuterol, cromolyn sodium and ipratropium bromide on the airway and circulating mediator responses to allergen bronchial provocation in asthma. Am Rev Respir Dis 1985; 132:986-92.

87. Anderson SD, Bye PIP, Schoeffel RE, Seale JP, Taylor KM, Ferris L. Arterial plasma histamine levels at rest, during and after exercise in patients with asthma. Effects of terbutaline aerosol. Thorax 1981; 36:259-67.

88. Peel ET, Gibson GJ. Effect of long-term inhaled salbutamol therapy on the provocation of asthma by histamine. Am Rev Respir Dis 1980; 121:973-8.

89. Harvey JE, Tattersfield AE. Airway response to salbutamol: effect of regular salbutamol inhalations in normal, atopic, and asthmatic subjects. Thorax 1982; 37:280-7.

90. Holtzman MJ, Cunningham JH, Sheller JR, Irsigler GB, Nadel JA, Boushey HA. Effect of ozone on bronchial reactivity in atopic and non-atopic subjects. Am Rev Respir Dis 1979; 120:1059-67.

91. Gayrard P, Orehek J, Charpin J. The prevention of the bronchoconstrictor effects of deep inspiration or of cigarette smoking in asthmatic patients by SCH 1000. Postgrad Med J 1975; 51 (suppl 7):102.

92. Widdicombe JG, Kent DC, Nadel JA. Mechanisms of bronchoconstriction during inhalation of dust. J Appl Physiol 1962; 17:613-6.

93. Simonsson BG, Jacobs FM, Nadel JA. Role of autonomic nervous system and the cough reflex in the increased responsiveness of airways in patients with obstructive airway disease. J Clin Invest 1967; 46:1812-8.

94. Tattersfield AE. Effect of beta-agonists and anticholinergic drugs on bronchial reactivity. Am Rev Respir Dis 1987; 136 (suppl):S64-8.

95. Gross NJ, Skorodin MS. Anticholinergic, antimuscarinic bronchodilators. State of the art. Am Rev Respir Dis 1984; 129:856-70.

96. Tashkin DP, Katz RM, Kerschner H, Rachelefsky GS, Siegel SC. Comparison of aerosolized atropine, isoproterenol, disodium cromoglycate, and placebo in the prevention of exercise induced asthma. Ann Allergy 1977; 39:311-8.

97. Gross NJ, Barnes PJ. A short tour around the muscarine receptor. Am Rev Respir Dis 1988; 138:765-7.

98. Faulkner D, Fryer AD, MacLagan J. Postganglionic muscarinic inhibitory receptors in pulmonary parasympathetic nerves in the guinea-pig. Br J Pharmacol 1986; 88:181-7.

99. Blaber LC, Fryer AD, MacLagan J. Neuronal muscarinic receptors attenuate vagally-induced contraction of feline bronchial smooth muscle. Br J Pharmacol 1985; 86:723-8.

100. Newcomb R, Tashkin DP, Hui KK, Conolly ME, Lee E, Dauphinee B. Rebound hyperresponsiveness to muscarinic stimulation after chronic therapy with an inhaled muscarine antagonist. Am Rev Respir Dis 1985; 132:12-5.

101. Koëter GH, Meurs H, Jonkman JHG, De Vries K. Protective effect of choline theophyllinate on histamine, acethylcholine, and propanolol-induced airflow obstruction. Respiration 1984; 45:139-46.

102. Cartier H, Lemire I, L'Archeveque J, Ghezzo H, Martin R, Malo JC. Theophylline partially inhibits bronchoconstriction caused by inhaled histamine in subjects with asthma. J Allergy Clin Immunology 1986; 77:570-5.

103. Levene S, McKenzie. Protective effect of theophylline on histamine-induced bronchoconstriction in asthmatic children. Br J Clin Pharmac 1986; 21:445-9.

104. McWilliams BC, Menendez R, Kelly AW, Mowick J. Effects of theophylline on inhaled methacholine and histamine in asthmatic children. Am Rev Respir Dis 1984; 130:193-7.

105. Ellis EF. inhibition of exercise-induced asthma by theophylline. J Allergy Clin Immunol 1984; 73:690-3.

106. Fairshter RD, Busse WW. Theophylline- How much is enough. J Allergy Clin Immunol 1986; 77:646-8.

107. Klein JJ, Lefkowitz MS, Spector SL, Chemiack RM. Relationship between serum theophylline levels and pulmonary function before and after inhaled beta-agonists in "stable" asthmatics. Am Rev Respir Dis 1983; 127:413-6.

108. Magnussen H, Reuss G, Jorres R. Theophylline has a dose-related effect on the airway response to inhaled histamine and methacholine in asthmatics. Am Rev Respir Dis 1987; 136:1163-7.

109. Persson CGA. Experimental lung action of xanthines. In: Andersson KE, Persson CGA (eds.). Anti-asthma xanthines and adenosine. Amsterdam: Exerpta Medica 1985:61-82.

110. Bergstrand H. Xanthines as phosphodiesterase inhibitors. In: Andersson KE, Persson CGA (eds.). Anti-asthma xanthines and adenosine. Amsterdam: Exerpta Medica 1985:16-22.

111. Ruffin RE, Newhouse MT. Dipyridamole is it a bronchodilator? Eur J Respir Dis 1981; 62: 123-6.

112. Higbee MD, Kuman M, Galant SP. Stimulation of endogenous catecholamine release by theophylline; a proposed additional mechanism of action for theophylline. J Allergy Clin Immunol 1982; 70:377-81.

113. Cushley MJ, Tattersfield AE, Holgate ST. Adenosine-induced bronchoconstriction in asthma. Am Rev Respir Dis 1984; 129:380-3

114. Kolbeck RC, Spier WA, Carrier GO, Bransome ED. Apparent irrelevance of cyclic nucleotides to the relaxation of tracheal smooth muscles induced by theophylline. Lung 1979; 156: 173-83.

115. Lichtenstein LM, Margolis S. Histamine release *in vitro*: inhibition by catecholamines and methylxanthines. Science 1968; 161:902-3.

116. Pauwels R, Van Reuterghem D,Van der Straeten M, Johanesson N, Persson CGA. The effect of theophylline and enprofylline on allergen-induced bronchoconstriction. J Allergy Clin Immunol 1985; 96:583-90.

117. Hendeles L, Weinberger M. Drugs in perspective: Theophylline, a state of the art review. Pharmacotherapy 1983; 3:2-44.

118. Jusko WJ, Gardner MJ, Mangione A, Schentag JJ, Koup JR, Vance JW. Factors affecting theophylline clearances: Age, tobacco, marijuana, cirrhosis, congestive heart failure, obesity, oral contraceptives, benzodiazepines, barbiturates and ethanol. J Pharm Sci 1979; 68: 1358-66.

119. Jonkman JHG, Upton RA. Pharmacokinetic drug interactions with theophylline. Clinical Pharmacokinetics 1984: 9:309-34.

120. Piafsky KM, Sitar DS, Rangho RE, Ogilvie RI (1977). Theophylline disposition in patients with hepatic cirrhosis. N Engl J Med 296:1495-7.

121. Persson CGA. Subdivision of xanthines. In: Andersson KE and Persson CGA (eds.). Anti-asthma xanthines and adenosine, Amsterdam, Excerpta Medica 1985:23-39.

122. Holgate ST, Mann JS, Cushley MJ. Adenosine as a bronchoconstrictor mediator in asthma and its antagonism by methylxanthines. J Allergy Clin Immunol 1984; 74:302-6.

123. Persson CGA, Kjellin G (1981). Enprofylline,a principally new antiasthmatic xanthine. Acta Pharmacol Tox 49:313-6.

124. Laursen LC, Johannsson N, Fagerström PO, Weeke B. Intravenous administration of enprofylline to asthmatic patients. Eur J Clin Pharmacol 1983; 24:323-7.

125. Laursen LC, Johannsson N, Weeke B. Continuous treatment of asthmatic patients with enprofylline and theophylline. Eur J Respir Dis 1984; 65:504-8.

126. Borgå O, Anderson KE, Edholm LE, Fagerström PO, Lunell E, Persson CGA. Pharmacokinetics of enprofylline in healthy subjects after intravenous single dose administration. Clin Pharmacol Ther 1983: 34:799-804.

127. Lunell E, Borgå O, Larsson R. Pharmacokinetics of enprofylline in patients with impaired renal function after a single dose intravenous dose. Eur J Clin Pharmacol 1984: 26:87-93.

128. Towart R, Rounding HP. Calcium antagonists and airways smooth muscle. In: Asthma; Clinical pharmacology and therapeutic progress. Kay AB (ed.) Blackwell Scientific Publications Oxford 1986.130-47.

129. Barnes PJ, Wilson NM, Brown MJ. A calcium antagonist, nifedipine, modifies exercise induced asthma. Thorax 1981; 36:726-30.

130. Patel KR. Calcium antagonists in exercise-induced asthma. Br Med J 1981; 282:932-3.

131. Cerrina J, Denjean A, Alexandre G, Lockhart A, Duroux P. Inhibition of exercise-induced asthma by a calcium antagonist, nifedipine. Am Rev Respir Dis 1981; 123:156-60.

132. Hendersen AF, Heaton RW, Costello JF. Effect of nifedipine on bronchoconstriction induced by inhalation of cold air. Thorax 1983; 38:512-5.

133. Malik S, O'Reilly J, Sudlow MF. Effect of sublingual nifedipine on inhaled histamine and methacholine-induced bronchoconstriction in atopic subjects. Thorax 1982; 37:230.

134. Williams DO, Barnet PJ, Vickers HP, Rudolp M. Effect of nifedipine on bronchomotor tone and histamine reactivity in asthma. Br Med J 1981; 283:348.

135. Fish JE, Norman PhS. Effects of the calcium channel blocker, verapamil, on asthmatic airway responses to muscarinic, histaminergic, and allergic stimuli. Am Rev Respir Dis 1986; 133: 730-4.

136. Patel KE. The effect of verapamil on histamine and methacholine-induced bronchoconstriction. Clin Allergy 1981; 11:441-7.

137. Popa VT, Somani P, Vimon V. The effect of inhaled verapamil on resting bronchial tone and airway contractions induced by histamine and acethylcholine in normal and asthmatic subjects. Am Rev Respir Dis 1984; 130:1006-13.

138. Metz SA. Anti-inflammatory agents as inhibitors of prostaglandine synthesis in man. Med Clin North Am 1981; 65:713-55.

139. Fairfax AJ. Inhibition of the late asthmatic response to house dust mite by non-steroidal anti-inflammatory drugs (case report). Prostaglandins Leukotrienes Med 1982; 8:239-48.

140. Shephard EG, Malan L, MacFarlane CM, Mouton W, Joubert JR. Lung function and plasma levels of tromboxane B_2, 6-ketoprostaglandin F_{1a} and B-thromboglobulin in antigen induced asthma before and after indomethacin pretreatment. Br J Clin Pharmacol 1985; 19:459-70.

141. Kirby JG, Hargreave FE, O'Byrne PM. Indomethacin inhibits allergen-induced airway hyperresponsiveness but not allergen-induced asthmatic responses (abstract). Am Rev Respir Dis 1987; 135 (suppl): A312

142. O'Byrne PM, Walters EH, Aizawa H, Fabbri LM, Holtzman MJ, Nadel JA. Indomethacin inhibits the airway hyperresponsiveness but not the neutrophil influx induced by ozone in dogs. Am Rev Respir Dis 1984; 130:220-4.

143. Walters EH. Effect of inhibition of prostaglandin synthesis on induced bronchial hyperresponsiveness. Thorax 1983; 38:195-9.

144. Cummings NP, Morris HG, Strunk RC. Failure of children with asthma to respond to daily aspirin therapy. J Allergy Clin Immunol 1983; 71:245-9.

145. Farr RS, Spector SL, Wangaard C. Asthma and nonsteroidal anti-inflammatory drugs. Ann Intern Med 1978; 89:577.

CHAPTER 3

AIMS OF THE STUDIES

The effect of anti-asthmatic drugs on BHR may be immediate, sustained, or may occur only in the long run after maintenance treatment.

Bronchodilators, such as beta-agonists and xanthine derivatives generally have a, sometimes pronounced, reducing effect on BHR. This effect is generally of short duration, but it can be sustained by repeated administration. "Prophylactic" anti-allergic drugs may have a reducing effect on BHR after long-term treatment.

This thesis deals with the immediate effect of the xanthine derivates theophylline and enprofylline. In addition, the long-term effect of the beta-adrenergic drug terbutaline and the corticosteroid budesonide on BHR were investigated.

The protective effect of orally administered xanthine derivatives and beta-agonists is less than that of inhaled bronchodilators, but the availability of sustained release preparations offers the opportunity to achieve a prolonged protective effect, without the necessity of frequent administration. Because the effect of theophylline and enprofylline is probably strongly dependent on adequate plasma concentrations, knowledge about pharmacokinetic properties and dose-response relations of these drugs is important.

The protective effect of bronchodilators like beta-agonists and xanthines is probably caused by functional antagonism, and it is thought that bronchodilators have no permanent influence on the pathophysiology of BHR. However, few experimental data are available on the effect on BHR of long-term treatment with these drugs. Beta-adrenergic drugs are widely used in mild asthmatic subjects, often as a single drug therapy. These drugs are very effective in the immediate relief of symptoms. Prolonged treatment with beta-adrenergic drugs may lead to beta-receptor desensitization, as has been demonstrated in several target tissues (see Chapter 1). Whether this effect might lead to a change in bronchial responsiveness is unknown.

BHR is often "divided" into a primary component, which is probably an endogenous defect, and a secondary part, induced by exogenous factors such as allergen exposure, which is variable in time and is superimposed on the primary part. Thus in an allergic asthmatic patient, this secondary hyperresponsiveness is predominantly caused by ongoing allergic (inflammatory) reactions. It is possible that at least this part of BHR can be treated with prophylactic, anti-inflammatory drugs, such as corticosteroids. Such drugs may decrease BHR more permanently than bronchodilators. Prolonged treatment is probably necessary to obtain a possible decrease in BHR, but the effect of duration of treatment or of dose of corticosteroids on the final result is unknown.

In addition to bronchoprotective studies with theophylline and enprofylline, pharmacokinetic studies with these drugs were carried out for the following reasons.

Theophylline has the disadvantage of a rather narrow therapeutic index, and it is necessary to keep a certain margin of safety to avoid side effects. Moreover, the pharmacokinetic behaviour of theophylline shows large interindividual variation, and is influenced by

many factors such as age, drug interactions, and disease states, such as liver cirrhosis and heart failure. Therefore adequate dosaging of theophylline is impossible without (sometimes frequent) monitoring of plasma concentrations. The recently developed xanthine derivative enprofylline is 3-5 times more potent as a bronchodilator than theophylline. It lacks the serious side effects of theophylline, such as seizures, whereas other side-effects such as nausea and headache occur with equal frequency. Enprofylline has a different pharmacokinetic profile, and is excreted, mainly unmetabolized, by the kidney with a short plasma half-life. Its clearance has been reported to be correlated to the creatinine clearance, which might make it, at least to some extent, predictable if information about renal function is available. If, moreover, the elimination rate of enprofylline during acute disease states could be shown to be more stable than that of theophylline, treatment with enprofylline might be safer than with theophylline, especially in acute disease states.

It was the purpose of the clinical studies described in chapters 4,5 and 6 to obtain more detailed knowledge of the pharmacokinetic behaviour of the new xanthine derivative enprofylline as compared with theophylline, in disease (liver cirrhosis, chronic renal failure, acute exacerbation of chronic airflow obstruction), and in an experimental intravenous dosing model.

In Chapter 4, the pharmacokinetic behaviour of theophylline and enprofylline in the course of treatment of an acute exacerbation of chronic obstructive lung disease was studied. The study was carried out to test the possibility that the elimination of enprofylline in acutely ill patients with complications like heart failure and hypoxaemia is (temporarily) decreased, as was demonstrated previously for theophylline. An attempt was made to identify clinical factors that might influence the pharmacokinetics of both drugs.

In Chapter 5, the pharmacokinetic behaviour of theophylline and enprofylline in patients with liver cirrhosis and patients with chronic renal failure was studied. The study was undertaken to examine the impact of these two disorders on the pharmacokinetic behaviour of the two drugs. Furthermore, the predictive value of liver function tests and renal function tests for the pharmacokinetics of the two drugs was assessed.

In Chapter 6 an intravenous infusion method is described which makes it possible to create instantaneous, stable plasma drug concentrations at desired levels. The infusion system delivers the drug at an exponentially decreasing rate. This study was carried out to investigate the possibility of rapidly obtaining stable plasma concentrations of theophylline and enprofylline on step-wise increasing levels.

In the second part of the clinical studies (Chapters 7, 8, 9 and 10) the acute and long-term effects of anti-asthmatic drugs on pulmonary function and BHR are described.

In Chapter 7, the protective effect of step-wise increasing plasma concentrations of theophylline and enprofylline (obtained with the infusion method described in Chapter 6) on methacholine-induced bronchoconstriction in asthmatic subjects was studied. The bronchodilatory and bronchoprotective effects of both drugs are related to their plasma concentrations. The purpose of this study was to determine the plasma concentration at which an optimal bronchoprotective effect (without side-effects) might be obtained.

Chapter 8 describes a crossover study which compares the effect on BHR of long-term treatment (4 weeks) with an inhaled corticosteroid, budesonide, with that of an inhaled

beta-agonist, terbutaline, in patients with allergic asthma. BHR was measured by repeated histamine and propranolol inhalation provocations (every 2 weeks). The study was carried out to test the possibility that treatment with a prophylactic anti-inflammatory drug might diminish BHR in allergic asthma. Such an effect would not be expected to occur after maintenance treatment with an inhaled beta-adrenergic drug. The effect of the two drugs on lung function, peak flow rates, symptom scores and blood eosinophils was also measured.

In Chapter 9, the influence of dosage and duration of treatment with inhaled budesonide on BHR (measured as methacholine PC_{20}) was studied. Two parallel groups of patients with allergic asthma were treated for 8 weeks with budesonide in a dosage of 200 μg or 800 μg/day. In addition we investigated whether it was possible to predict the change in BHR induced by budesonide from pretreatment patient characteristics, such as initial methacholine PC_{20}, serum IgE concentration, or the number of eosinophils in peripheral blood.

Chapter 10 deals with the influence of long-term treatment with budesonide on the configuration of the maximal expiratory flow-volume curve. This was compared with the effect after instantaneous bronchodilatation, obtained after inhalation of ipratropium bromide.

Even in patients with mild bronchial obstruction, the flow-volume curve tends to be convex towards the volume axis. The exact pathophysiological basis of the increased curvilinearity of the MEFV-curve is unknown. It may be caused by preferential obstruction of peripheral airways, or by regional inhomogeneity of the forced expiratory flow. This abnormality seems to be a very sensitive parameter of mild bronchial obstruction.

In mild asthmatic subjects, an instantaneous improvement of the FEV_1 to the predicted value was seen after administration of a bronchodilator; such an improvement can also be obtained after prolonged treatment with a corticosteroid. The study was carried out to test the possibility that parameters describing the curvilinearity of the MEFV-curve can show differences between instantaneous bronchodilatation and bronchodilatation resulting from anti-inflammatory treatment during several weeks.

CHAPTER 4

THE PHARMACOKINETICS OF THEOPHYLLINE AND ENPROFYLLINE IN THE ACUTE AND THE RECOVERY PHASE OF EXACERBATIONS OF CHRONIC OBSTRUCTIVE LUNG DISEASE

J Kraan[1], GH Koëter[1], ThW van der Mark[2], M Boorsma[3], OE de Noord[4], and JHG Jonkman[4]

[1]Department of Pulmonology, [2]Department of Lung Function, University of Groningen, [3]Astra Pharmaceutica BV, and [4]Pharma Bio-Research International BV, Assen, the Netherlands.

Summary

The pharmacokinetic behaviour of theophylline and enprofylline was examined in patients with an acute exacerbation of chronic obstructive lung disease (COLD). Enprofylline is a new xanthine derivative, which is excreted, unmetabolized, by the kidney. The study was carried out in a group of eight patients with uncomplicated COLD, and a group of ten patients with COLD complicated by hypoxaemia or heart failure. The elimination constant (K_{el}) was determined in the acute phase and during recovery after 6 days of treatment. During continuous treatment in the intermediate period, the total body clearance of the xanthine was determined daily.

There was a wide interindividual variation of elimination for both drugs, especially in the patient group with complicated COLD. In the acute phase, the elimination of both drugs was considerably decreased in the patients with COLD complicated by hypoxaemia or heart failure, compared with the patient group with uncomplicated COLD. However, there was a partial recovery of the elimination parameters during clinical improvement of the patients. The decreased enprofylline clearance in the acute phase may have been caused by decreased renal function in the phase of (severe) hypoxaemia. During recovery of the patients, the clearance of enprofylline was significantly correlated to the creatinine clearance.

Although it may be easier to give rough guidelines about initial treatment (e.g. on basis of serum creatinine values) for enprofylline than for theophylline, it is our opinion, that plasma concentrations should be frequently measured during maintenance treatment with both xanthine drugs, in view of the many factors that influence drug clearance especially in acute illness.

Introduction

Theophylline is commonly administered intravenously to achieve bronchodilatation in patients with an exacerbation of chronic obstructive lung disease (COLD). This drug has a rather narrow therapeutic range: plasma concentrations below 5 $mg.L^{-1}$ are considered ineffective[1] and plasma concentrations above 20 $mg.L^{-1}$ are potentially toxic[2]. The pharmacokinetic behaviour of theophylline (metabolized by the liver) shows large interindividual variation and is influenced by many factors, such as age[3] and drug interaction[4]. In acute disease states like pulmonary oedema[5] and right heart failure[6], a significant decrease in theophylline clearance has also been reported. Therefore, monitoring of plasma concentrations is considered necessary during treatment with theophylline[7].

Enprofylline is a recently developed xanthine derivative, with a bronchodilatory potency 4-5 times greater than that of theophylline[8]. Enprofylline is rapidly excreted, mainly unmetabolized, by the kidney[9]. In recent studies its clearance has been reported to be correlated to the creatinine clearance[10,11] and thus, at least to some extent, predictable if the creatinine clearance is known. If, moreover, the elimination rate of enprofylline during acute disease states could be shown to be more stable than that of theophylline, intravenous treatment of acute bronchial obstruction with enprofylline might be safer than with theophylline.

We studied the pharmacokinetics of theophylline and enprofylline in patients hospitalized for an acute exacerbation of COLD. The study was carried out in a subgroup of patients with uncomplicated COLD and normal arterial blood gases, and in a subgroup of patients with airflow obstruction complicated by arterial hypoxaemia and/or heart failure.

Aims of the study were to compare the pharmacokinetic behaviour of theophylline and enprofylline in the course of treatment of an acute exacerbation of obstructive lung disease, and to identify clinical factors that might influence the pharmacokinetics of these drugs.

Patients and methods

Patients

Eighteen adult patients, admitted to the hospital for treatment of an acute exacerbation of COLD, entered the study. The patients were divided into two subgroups. Subgroup I (8 patients) consisted of COLD patients with normal arterial blood gases. Subgroup II (10 patients) consisted of patients with severe arterial hypoxaemia (P_aO_2 less than 7.0 kPa) and/or clinical signs of left or right heart failure.

Patient characteristics are given in table I. All patients had a history of dyspnoea and wheezing. Most patients had a history of chronic cough and recurrent bronchial infections. Two of the patients were smokers, 11 patients were ex-smokers. Eleven patients used oral theophylline (6 patients in subgroup I and 5 patients in subgroup II) in addition to various other kinds of (inhaled) anti-asthmatic drugs. Lung function values, measured when the patients were in a stable condition either at the end of their hospitalization or on a separate occasion, are also given in table I. The values of slow inspiratory vital capacity (VC) and FEV_1 are expressed as percentage of the predicted value[12]. In general, the pa-

Table 1. Clinical data.

	Subject no	Age (yr)	Weight (kg)	VC (%pred.)	FEV_1 (%pred.)	Creatinine Clearance ($mL.h^{-1}.kg^{-1}$)	Creatinine Serum ($\mu mol.L^{-1}$) day 1	Creatinine Serum ($\mu mol.L^{-1}$) day 7	P_aO_2 (kPa) 0h	P_aO_2 (kPa) 3h	P_aO_2 (kPa) day 6
Subgroup I											
	1	67	42	83	57	77	55	54	10.8	10.0	-
	2	70	81	61	59	62	92	84	10.0	9.8	-
	3	58	75	104	61	58	76	97	7.4	11.3	-
	4	19	68	83	50	116	76	69	8.8	9.5	-
	5	22	53	79	53	99	86	78	9.4	10.0	-
	6	63	80	96	97	81	85	74	14.0	13.8	-
	7	64	65	96	104	58	83	72	9.3	10.6	-
	8	62	90	100	48	76	74	73	10.3	12.2	-
mean		53	69	88	66	78	80	75	10.0	9.9	-
SEM		±7	±6	±5	±8	±7	±4	±4	±0.7	±0.5	-
Subgroup II											
	9	52	67	48	20	106	59	51	5.5	7.2	7.2
	10	62	59	47	27	79	71	69	6.1	7.1	10.2
	11	63	54	68	65	59	127	95	11.6	11.0	10.8
	12	56	52	70	55	66	84	82	5.0	7.2	8.8
	13	71	65	71	44	69	92	92	5.6	6.7	8.5
	14	46	73	71	53	73	98	92	7.2	10.2	11.0
	15	70	68	64	37	44	94	73	5.5	7.7	7.0
	16	71	68	82	51	56	112	106	9.1	8.9	10.8
	17	72	66	50	20	58	81	65	6.0	8.2	8.3
	18	72	55	52	34	64	94	85	6.1	10.5	10.0
mean		64	63	62*	41*	67	91	81**	6.8	8.5***	9.3‡
SEM		±3	±2	±4	±5	±5	±6	±5	±0.7	±0.5	±0.5

Subgroup I: Patients with uncomplicated exacerbation of COLD. Subgroup II: Patients with an exacerbation complicated by arterial hypoxaemia or congestive heart failure.
VC (slow inspiratory vital capacity) and FEV_1 were measured when the patients were in a stable condition.
P_aO_2: arterial PO_2 on admission, after 3 hours, and after 6 days of hospitalization. Creatinine clearance: mean value of 6 consecutive days. *: significant difference between subgroup I and II ($p<0.01$). **, ***, and ‡: significant difference from the pretreatment value (**: $p<0.05$, ***: $p<0.01$, ‡: $p<0.001$).
During the study patients no 2,5,6,7,10,11,13,15, and 16 were treated with theophylline, and patients no 1,3,4,8,9,12,14,17, and 18 were treated with enprofylline.

tients in subgroup II had, more severe COLD than the patients in subgroup I. Patients with known liver or renal disease, or patients who used medication known to interfere with the pharmacokinetics of theophylline or enprofylline, were excluded from the study.

Study design

The decision to admit the patients and to treat them with intravenous drugs was taken by a pulmonary physician, who did not cooperate in the study. All patients gave informed consent to participate in the study.

After admission to the hospital, 2 intravenous catheters (one in the left and one in the right arm) were inserted, one for infusion of the drugs with a constant infusion pump (Ivac), and one on the opposite side for taking blood samples.

An arterial blood sample was taken while the patient was breathing room air, and a second sample was taken 3h after starting treatment (including oxygen via a nasal catheter at 1 $L.min^{-1}$, in case of a room air P_aO_2 less than 9.0 kPa). A third sample was taken on day 6 (while the patients to whom oxygen was administered initially continued oxygen treatment).

The patients were randomized to treatment with either theophylline or enprofylline in an open study design. The treatment and blood sampling protocol is summarized in figure 1. On admission the patients received a loading dose during a 30-minute intravenous infusion (theophylline 5 $mg.kg^{-1}$ (2.5 $mg.kg^{-1}$ if the patient had used oral theophylline) or enprofylline 1 $mg.kg^{-1}$). A continuous infusion (dosage, see below) was started after this loading infusion, (phase A,figure 1) lasting 6-18 hours depending on time of admission. After this phase (A), thus within 24 hours after admission, the infusion was discontinued temporarily, for a first determination of the plasma drug half-life (phase B). After this elimination phase, continuous infusion of the drug was restarted (phase C). The maintenance dosage of theophylline was 0.5 $mg.kg^{-1}.h^{-1}$ for the patients in subgroup I. For the patients in subgroup II, a lower (fixed) administration rate was chosen (0.25-0.40 $mg.kg^{-1}.h^{-1}$), because these patients were expected to have an impaired theophylline elimination. The enprofylline maintenance dosage was 0.5 $mg.kg^{-1}.h^{-1}$ for all patients. On day 7, drug infusion was discontinued for a second determination of plasma drug half-life (phase D).

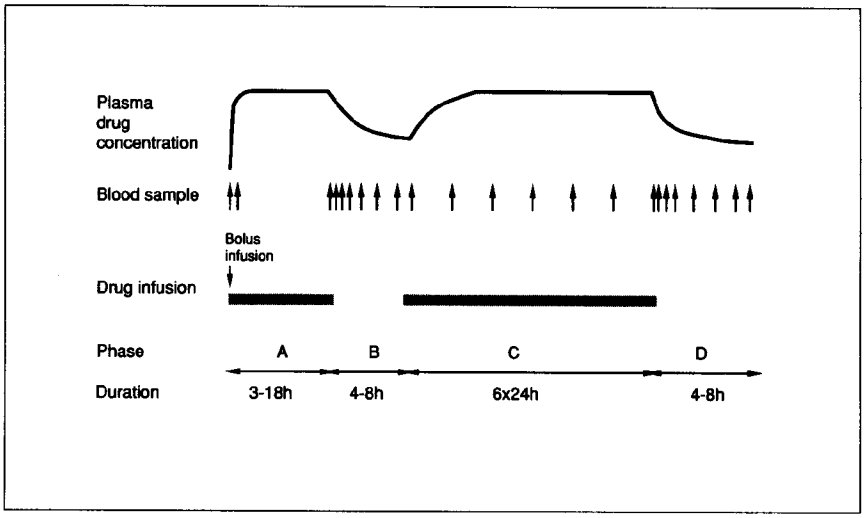

Figure 1. Study design (explanation: see text).

Besides to treatment with the xanthine drug, the patients were treated in an standardized way with prednisolone via continuous infusion (50 mg during the first 24 hours, tapering off the daily dose in accordance with the clinical status of the patient), and amoxycilline 375-500 mg 4 times daily. After the first 3 hours, inhaled bronchodilators were given additionaly in accordance with instructions of the pulmonary physician who treated the patients. The study protocol was approved by the hospital's medical ethics committee.

Pharmacokinetic determinations

During the 2 elimination periods (phase B and D), blood samples were taken at t= 0,0.5,1,1.5,2,3,4h (enprofylline) or at t= 0,0.5,1, 1.5,2,3,4,6,8h (theophylline) after discontinuing the drug infusion. The elimination constant (K_{el}) was calculated from the slope of the linear last part of the $\ln(C_p(t))$-time curve, where $C_p(t)$ is the plasma drug concentration at time t. During phase A and C, total body clearance (Cl) was estimated for daily intervals. For this purpose, daily blood samples were taken at 9.00 a.m.. The drug Cl was calculated from the equation:

$$Cl = \frac{K_o}{C_p(t)} \qquad (mL^{-1}.h^{-1}.kg^{-1}) \qquad (1)$$

where K_o is the actual infusion rate ($mg.kg^{-1}.h^{-1}$) during a time interval and $C_p(t)$ is the plasma drug concentration at the end of the time interval. These calculations can be performed on the assumption that an approximately steady state, a steady state exists during the time interval. In some patients, the initial time intervals after starting the continuous infusion were too short in relation to the plasma drug half-life ($t_{1/2}$, estimated during phase B) to assume the presence of a steady state. If these time intervals were less than 2 times $t_{1/2}$, Cl was calculated from the equation:

$$Cl = \frac{K_o.(1-e^{-K_{el}.t})}{C_p(t)-C_p(0).e^{-K_{el}.t}} \ (mL^{-1}.h^{-1}.kg^{-1}) \qquad (2)$$

where $C_p(0)$ is the plasma drug concentration at the start of the interval[13]. For K_{el}, the value calculated from elimination phase B was used.

During maintenance treatment, the administration rates of theophylline were somewhat different in the patients of the two clinical subgroups (see study design). Yet to be able to estimate the consequences of a standardized dosing regime, we calculated the plasma concentrations that would result, if the dosage regime had been similar for all patients, as 0.5 $mg.kg^{-1}.h^{-1}$. For these calculations we used equation 1 and the Cl values of theophylline during maintenance infusion.

Statistical comparison of pharmacokinetic values between the clinical subgroups and comparisons of values during the acute and recovery phase within the patient subgroups were carried out using Student's t-tests.

Theophylline and enprofylline assay

Blood was collected in heparinized tubes and centrifuged immediately. Plasma was separated and stored at -20° C until analysis. Drug analysis was carried out with high pressure liquid chromatography[14,15].

Renal function

The serum creatinine concentration and the amount of creatinine in the 24 hours urine were measured daily for the calculation of the creatinine clearance. During the initial phase of hospitalization, the urine collection from the acutely ill patients was sometimes incomplete. For this reason the calculated creatinine clearance during the first 24 hours was not reliable in all cases. Therefore, the change in renal function during the study period was estimated from the daily determined serum creatinine concentration.

Results

Theophylline pharmacokinetics

In Table 2 the mean values of K_{el} and Cl (group means and total mean (SEM)) during the acute and recovery phase are shown. The individual K_{el} values are shown in Figure 2. There is a large interindividual variation of K_{el} and Cl. During the acute phase, the mean value of K_{el} in subgroup I was significantly higher than in subgroup II ($p<0.05$). In subgroup I, both K_{el} and Cl were quite stable, while in subgroup II K_{el} and Cl tended to increase (n.s.) during the study period.

Enprofylline pharmacokinetics

In Table 3 the mean values of K_{el} an Cl (group means and total mean (SEM)) during the acute and recovery phase are shown. The individual K_{el} values are shown in Figure 3. The

Table 2. Pharmacokinetic parameters of theophylline during acute and recovery phase of an exacerbation of chronic airflow obstruction.

	Acute phase	Recovery phase	Acute phase	Recovery phase
	K_{el} (h^{-1})		Cl ($mL.kg^{-1}.h^{-1}$)	
Subgroup I (n=4)	0.100 ±0.008	0.098 ±0.007	37 ±2	37 ±3
Subgroup II (n=5)	0.056 * ±0.011	0.078 ±0.018	25 ±4	30 ±5
All patients (n=9)	0.075 ±0.010	0 087 ±0.011	30 ±3	33 ±3

Subgroup I and II: Patients with uncomplicated and complicated COLD respectively. K_{el}: elimination constant. Cl: theophylline total body clearance. Group and total mean values ±SEM are given. *: significant difference between subgroup I and II ($p<0.05$).

Table 3. Pharmacokinetic parameters of enprofylline during acute and recovery phase of an exacerbation of chronic airflow obstruction.

	Acute phase	Recovery phase	Acute phase	Recovery phase
	K_{el} (h^{-1})		Cl ($mL.kg^{-1}.h^{-1}$)	
Subgroup I (n=4)	0.368 ±0.029	0.341 ±0.031	185 ±46	191 ±39
Subgroup II (n=5)	0.178* ±0.025	0.269** ±0.032	93 ±11	133 ±19
All patients (n=9)	0.262 ±0.038	0.301 ±0.025	134 ±25	159 ±21

Subgroup I and II: Patients with uncomplicated and complicated COLD respectively. K_{el}: elimination constant. Cl enprofylline total body clearance. Group and .total mean values ±SEM are given. *: significant difference between subgroup I and II ($p< 0.005$), **: significant difference between acute and recovery phase ($p<0.01$).

initial values of both parameters are higher in subgroup I than in sub-group II($p<0.005$). In subgroup I the parameters remained stable in the recovery phase, but in subgroup II there was an increase of K_{el} ($p<0.01$) and Cl (n.s., $p=0.06$).

Clinical parameters (Table I)

The mean P_aO_2 values on admission of the patients in subgroup I and II were 10.0±0.7 and 6.8±0.7 kPa, respectively ($p<0.005$). There was a significant improvement of P_aO_2 in the patients in subgroup II ($p<0.01$), due to treatment with oxygen, while there was no change in P_aO_2 in subgroup I. There was no difference between patients treated with theophylline or enprofylline with respect to changes in P_aO_2.

The mean creatinine clearance in the patients in subgroup II was lower than in subgroup I (n.s.). In subgroup I, the serum creatinine concentration did not change significantly, while in subgroup II there was a significant decrease of serum creatinine concentrations ($p<0.05$, day 1 compared with day 6), indicating that renal function had improved during the treatment period.

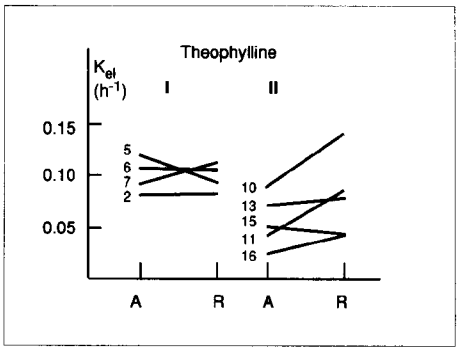

Figure 2. Theophylline K_{el} during acute (A) and recovery (R) phase of an exacerbation of chronic obstructive lung disease. I: subgroup I; II: subgroup II. Individual data; the subject numbers are the same as in table I. Mean values are given in table II.

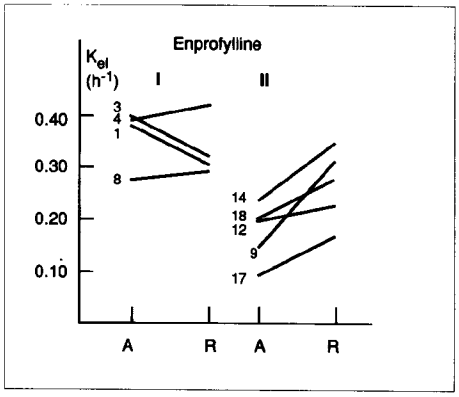

Figure 3. Enprofylline K_{el} during acute (A) and recovery (R) phase of an exacerbation of chronic obstructive lung disease. I: subgroup I; II: subgroup II. Individual data; the subject numbers are the same as in table I. Mean values are given in table III.

In two recent studies[10,11], a significant correlation was found between the enprofylline Cl and creatinine Cl in clinically stable patients with renal dysfunction and in healthy subjects. The regression line and the confidence limits calculated from the combined data of these two studies are shown in figure 4. The values of creatinine Cl compared with enprofylline Cl of the nine patients treated with enprofylline in the present study are plotted in this figure as well. For each of these patients the mean value of the creatinine and enprofylline clearance on day 4, 5, and 6 was calculated. Most of the plotted points are within the confidence limits calculated in the previous studies.

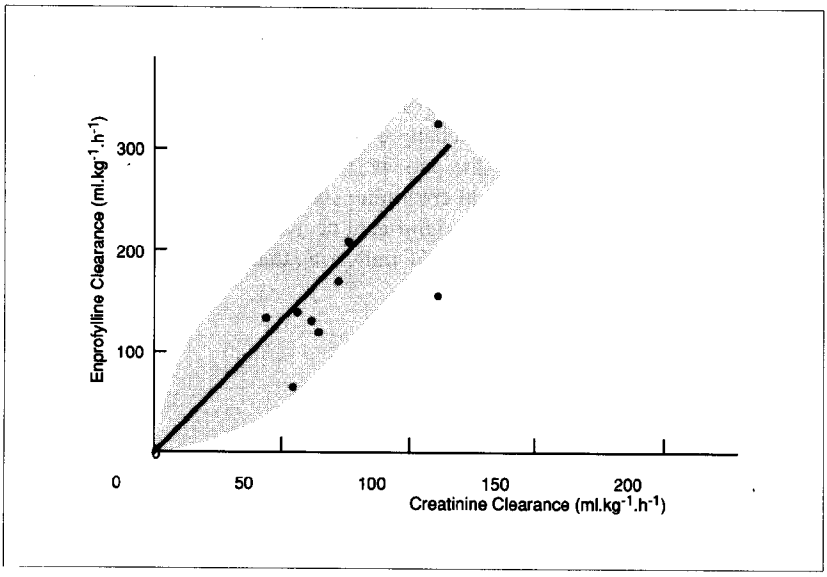

Figure 4. Correlation between enprofylline CL and creatinine CL.
The regression line and the confidence limits as calculated from two previous studies in healthy subjects, and patients with chronic renal disease (10,11) were drawn. The points refer to the individual data of the patients in the present study, while they were in the stable recovery phase.

Pharmacokinetic behaviour of theophylline and enprofylline and consequences for plasma drug concentration during continuous infusion

For each patient we counted the treatment days during which the steady state xanthine plasma concentrations fell outside the defined therapeutic range (theophylline: 8-20 $mg.L^{-1}$, enprofylline 2-5 $mg.L^{-1}$). For enprofylline, the measured plasma concentrations were used, while for theophylline the steady state drug plasma concentration was calculated that would have resulted from a standardized continuous infusion (0.5 $mg.kg^{-1}.h^{-1}$, see methods, pharmacokinetic determinations). The number of treatment days on which the drug plasma concentration would have been above or below a defined therapeutic range is presented in table 4.

Table 4. Predicted theofylline and enprofylline plasma levels at steady state during continuous infusion treatment period.

	Theophylline % of treatment days		Enprofylline % of treatment days	
	8mg.L^{-1}	20mg.L^{-1}	2mg.L^{-1}	5mg.L^{-1}
Subgroup I	-	2/23	6/24	-
Subgroup II	-	12/28	-	7/29

In this table the number of treatment days with drug plasma concentration out of the range 8-20 $mg.L^{-1}$ (theophylline) or 2-5 $mg.L^{-1}$ (enprofylline) and the total number of days for each treatment in both patients groups are presented. The drug plasma concentrations for each treatment day are calculated values from the real plasma concentration, the real drug administration rate, drug clearance and the "normalized" drug administration rate (for both theophylline and enprofylline 0.5 $mg.h^{-1}.kg^{-1}$).

For both drugs, the predicted steady state plasma concentrations fell outside the defined therapeutic range on an important number of treatment days. In the patients with an uncomplicated exacerbation of airflow obstruction, the predicted theophylline steady state concen-tration was sometimes above the therapeutic range (7% of treatment days). Enprofylline plasma concentrations were below the therapeutic range on 25% of, the treatment days. In patients with hypoxaemia or heart failure, 43% and 27% of plasma concentrations of theophylline and enprofylline, respectively, were above the therapeutic range.

Side effects

Two patients treated with theophylline experienced slight side effects like headache and nausea in a phase in which they appeared to have plasma theophylline concentrations above 20 $mg.L^{-1}$.

Two patients treated with enprofylline experienced slight side effects (headache, palpitations, nausea) when their plasma enprofylline concentrations were in the range of 4-6 $mg.L^{-1}$. Two other patients who had plasma enprofylline concentrations above 6 $mg.L^{-1}$ did not experience any side effect. No serious side effects were noted during treatment with either drug.

Discussion

Both in the acute and in the recovery phase of an exacerbation of COLD, a large interindividual variation of theophylline and enprofylline elimination was observed. In patients with normal arterial blood gases, the K_{el} of both drugs in the acute phase was significantly larger than in patients with airflow obstruction complicated by heart failure or hypoxaemia. Moreover, in the patients with normal blood gases the elimination of both drugs remained stable during the treatment period. In patients with COLD complicated by hypoxaemia or heart failure, elimination of both enprofylline and theophylline increased in the recovery phase (enprofylline $p<0.01$, theophylline n.s.).

The large interindividual variation of theophylline elimination in the present study is in accordance with data in the literature[3,7]. There are conflicting reports about the stability of theophylline elimination in time, in healthy subjects or patients with stable COLD. Some authors found only small variations of elimination parameters in time[16,17], while others report that fluctuations of more than 30% are common[18].

In patients with bronchial obstruction complicated by left or right heart failure, theophylline elimination may be decreased temporarily[5,6,19]. Powell et al.[19] found that in patients with heart failure, the initially decreased theophylline Cl could increase by more than 100% when the patients improved clinically. Our results are in accordance with these data, although in our patients the changes of theophylline Cl in time were less pronounced, possibly due to incomplete recovery of some of the patients during the study period. As was concluded earlier by Powell[19], the temporary decrease in theophylline elimination might lead to potentially toxic plasma concentrations, if a standardized dosing schedule (e.g. 0.5 $mg.kg^{-1}.h^{-1}$) had been applied. Furthermore, the changes in theophylline elimination in time also make proper dosing difficult.

Our finding of decreased enprofylline elimination in the acute phase of bronchial obstruction in some patient groups has not been previously reported. Because enprofylline is mainly excreted unmetabolized by the kidney[9], we also paid attention to changes in renal function during the present study. Although the mean value of creatinine Cl in the acute phase was only slightly lower in subgroup II than in subgroup I, renal function appeared to improve significantly in this patient group during the recovery phase. These changes possibly reflect a decreased renal blood flow, which is known to occur in cor pulmonale[20] and hypoxaemia[21]. These changes in renal function (decreased creatinine Cl during the acute phase and increase in renal function during the recovery phase), may explain the changes observed in enprofylline Cl in the hypoxaemic patient group. On the other hand, it is also possible that hypoxaemia by itself has a decreasing effect on the active renal excretion of enprofylline.

We found that the mean value of enprofylline Cl for each patient was to some extent predictable from the creatinine Cl. Most creatinine Cl versus enprofylline Cl values fall within the confidence limits calculated for the relationship between creatinine Cl and enprofylline Cl in two previous studies[10,11]. Another study has shown that in patients with normal renal function, the interindividual variation in enprofylline elimination is less than in the case of theophylline[22].

We conclude from these results that when information about renal function is available and when enprofylline dosing is adapted to renal function, toxic plasma drug concentra-

tions may be more easily avoided with enprofylline than with theophylline. However, when a patient is hospitalized in the acute phase, renal function may be decreased and exact knowledge about this change cannot generally be obtained before treatment is started. Therefore, although it may be possible that rough guidelines about initial treatment (e.g. based on serum creatinine values) can be given more easily for enprofylline than for theophylline, we recommend that plasma concentrations are frequently measured during maintenance treatment with these two xantine drugs, in view of the many factors influencing drug clearance, especially in acute illness.

References

1. Mitenko PA, Ogilvie RI. Rational intravenous doses of theophylline. N Engl J Med 1973; 289:600-3.
2. Hendeles L, Bighley L, Richardson RH, Hepler CD, Charmichael J. Frequent toxicity from i.v. aminophylline infusions in critically ill patients. Drug Int Clin Pharm 1977; 11:12-7.
3. Jusko WJ, Gardner MJ, Mangione A, Schentag JJ, Koup JR, Vance JW. Factors affecting theophylline clearances: Age, tobacco, marijuana, cirrhosis, congestive heart failure, obesity, oral contraceptives, benzodiazepines, barbiturates and ethanol. J Pharm Sci 1979; 68: 1358-66.
4. Jonkman JHG, Upton RA. Pharmacokinetic drug interactions with theophylline. Clinical Pharmacokinetics 1984; 9:309-34.
5. Piafsky KM, Sitar DS, Rangho RE, Ogilvie RI. Theophylline kinetics in acute pulmonary edema. Clin Pharmacol Ther 1977; 21:310-6.
6. Vicuna N, McNay Jl, Ludden TM, Schwertner H. Impaired theophylline clearance in patients with cor pulmonale. Br J Clin Pharmac 1979; 7:33-7.
7. Hendeles L, Weinberger M. Drugs in perspective: Theophylline, a state of the art review. Pharmacotherapy 1983; 3:2-44.
8. Lunell E, Svedmyr N, Andersson KE, Persson CGA. A novel bronchodilator xanthine apparently without adenosine receptor antagonism and tremorogenic effect. Eur J Respir Dis 1983; 64:333-9.
9. Borgå O, Anderson KE, Edholm LE, Fagerström PO, Lunell E, Persson CGA. Pharmacokinetics of enprofylline in healthy subjects after intravenous single dose administration. Clin Pharmacol Ther 1983; 34:799-804.
10. Lunell E, Borgå O, Larsson R. Pharmacokinetics of enprofylline in patients with impaired renal function after a single dose intravenous dose. Eur J Clin Pharmacol 1984; 26:87-93.
11. Kraan J, Jonkman JHG, Borgå O, Ekman I, Koeter GH, de Zeeuw RA. Comparison of enprofylline and theophylline pharmacokinetics in patients with impaired hepatic or renal function. In: Andersson KE and Persson CGA eds., Anti-asthma xanthines and adenosine, Amsterdam, Excerpta Medica. 1985; 267-70.
12. Tammeling GJ. Standard values for lung volume and ventilatory capacity of sanatorium patients. Selected Papers. Royal Netherlands Tuberculosis Association 1961; 1:65.
13. Ritschel WA. Handbook of basic pharmacokinetics; second edition. Drug Intelligence Publications Inc., Hamilton 1980.

14. Jonkman JHG, Schoenmaker R, Greving JE, Zeeuw de RA. Rapid and selective theophylline serum and saliva assay by means of high pressure liquid chromatography. Pharm Weekblad (Sci Ed) 1980; 2:49-53.

15. Edholm LE, Bodenas C, Heintz L, Ogren L. Column liquid chromatography for the quantitive analysis of enprofylline in biological samples. In: Andersson KE and Persson CGA (eds.). Anti-asthma xanthines and adenosine, Amsterdam, Excerpta Medica. 1985; 238-42.

16. Jenne JW, Wyze E, Rood FS, McDonald FM. Application to adjustment of the clinical dose of aminophylline. Clin Pharmacol Ther 1972; 13:349-60.

17. Whyatt R, Weinberger M, Hendeles L. Oral theophylline dosage for the management of chronic asthma. J Pediatr 1978; 92:125-130.

18. Upton RA, Thiercelin JF, Guentert Th, Wallace SM, Powell JR, Samson L, Riegelman S. Intraindividual variability in theophylline pharmacokinetics: statistical verification in 39 of 60 healthy young adults. J Pharmacokin Biopharm 1982; 10:123-134.

19. Powell JR, Vozek S, Hopewell Ph, Costello J, Sheiner LB, Riegelman S. Theophylline disposition in acutly ill hospitalized patients; the effect of smoking, heart failure, severe airway obstruction and pneumonia. Am Rev Respir Dis 1978; 118:229-34.

20. Richens JM, Howard P. Oedema in cor pulmonale. Clin Sci 1982; 62:255-9.

21. Andersson RJ, Pluss RG, Pluss WT, Bell J, Zerbe GG. Effect of hypoxia and hypercapnic acidosis on renal autoregulation in the dog: role of renal nerves. Clin Sci 1983; 65:533-8.

22. Laursen LC, Johannesson N, Weeke B. Pharmacokinetics of theophylline and enprofylline in patients requiring a high or low dose of theophylline. Eur J Clin Pharmacol 1985; 29:115-7.

CHAPTER 5

THE PHARMACOKINETICS OF THEOPHYLLINE AND ENPROFYLLINE IN PATIENTS WITH LIVER CIRRHOSIS AND IN PATIENTS WITH CHRONIC RENAL DISEASE

J.Kraan[1], J.H.G.Jonkman[2,6], G.H.Koeter[1], C.H.Gips[3], P.E.de Jong[4], Th.W.van der Mark[5], I.Ekman[7], R.A.de Zeeuw[2].

[1]Department of Pulmonology, [2]of Toxicology, [3]of Hepatology, [4]of Nephrology, [5]of Lung Function, University of Groningen and [6]Pharma Bio-Research International B.V., Assen, the Netherlands, and [7]AB Draco, Lund, Sweden.

Summary

The pharmacokinetics of theophylline and enprofylline in patients with liver cirrhosis, patients with chronic renal failure, and healthy subjects have been studied. The predictive value of routine tests of liver function and renal function (creatinine clearance) for the theophylline and enprofylline total body clearances have been assessed.

Theophylline clearance was significantly decreased in the patients with liver cirrhosis compared with both the patients with renal failure and the healthy subjects (the mean values in the three groups were 24, 47, and 46 $mL.h^{-1}.kg^{-1}$ respectively).

Enprofylline clearance was significantly decreased in the patients with chronic renal failure, compared with both the patients with liver cirrhosis and the healthy subjects (the values in the three groups were 64, 250, and 289 $mL.h^{-1}.kg^{-1}$ respectively). There was a strong correlation between creatinine clearance and enprofylline clearance, while there was only a poor correlation between the liver function tests and theophylline clearance.

It appears that in various clinical situations, enprofylline elimination can be predicted more precisely than theophylline elimination, which may make the drug safer in clinical practice.

Eur J Clin Pharmacol 1988; 35:357-62

Introduction

Theophylline is one of the most widely used bronchodilating drugs. Its therapeutic efficacy is related to its plasma concentrations[1], but it has a rather narrow therapeutic range. Theophylline is mainly eliminated by biotransformation in the liver[2]. Its clearance varies widely interindividually, and is influenced by many factors such as age,drug interactions[3], and diseases such as congestive heart failure[4] and liver disease. Although in patients with liver disease, theophylline elimination can be severely diminished[5,6,7,8], routine liver function tests, such as clotting tests and the serum albumin concentration, are of limited value in predicting the degree of reduction[5,6]. Therefore, close monitoring of theophylline serum concentrations is indicated in these patients.

Enprofylline is a recently developed xanthine derivative with a bronchodilating potency four to five times greater than theophylline[9]. Although it may cause adverse effects such as nausea and headache, serious central nervous system actions have not been reported. It is mainly excreted unmetabolized via the kidney by active transport[10], and its half-life is short. The rate of its elimination is reduced in patients with renal impairment, and enprofylline clearance has been reported to be correlated with creatinine clearance[11,12]. Since it is easier to quantify renal than liver dysfunction, it is conceivable that dosage adjustment for enprofylline can be carried out appropriately on the basis of measurement of renal function.

We have studied the interindividual variation of the pharmacokinetics of both these drugs in patients with liver cirrhosis,patients with chronic renal failure and a group of healthy subjects. The study was also undertaken to compare the predictive value of liver function tests and renal function tests for the pharmacokinetic behaviour of theophylline and enprofylline respectively.

Patients and methods

Patients

We studied 7 patients with liver cirrhosis,7 patients with chronic renal failure and 10 healthy subjects. The clinical data are given in Table 1.

The diagnosis of liver cirrhosis was confirmed by laparoscopy and liver biopsy. Liver cirrhosis was not complicated by ascites in the studied patients. The liver patients had creatinine clearances above 70 $mL.h^{-1}.kg^{-1}$ and there were no signs of renal disease (e.g. proteinuria, abnormal urine sediment). Routine liver function tests showed varied abnormalities. Liver protein synthesis function was impaired, as reflected by the low plasma concentration of albumin (normal range 38-51 $g.L^{-1}$) and anti-thrombin III (AT_{III}, normal range 80%). None of these patients used diuretics or drugs that are known to interact with theophylline.

The patients with chronic renal failure had a creatinine clearance below 50 $mL.h^{-1}.kg^{-1}$ (range 5-46 $mL.h^{-1}.kg^{-1}$). Their disease was stable from 3 months before and during the study. They had no liver disease and routine blood chemical tests such as serum aspartate amino transferase (AsT, normal range 40 $iU.L^{-1}$), and serum bilirubin (range 3-25 $\mu mol.L^{-1}$) were within the normal range. All but one patient had normal serum alkaline phospha-

Table 1. Clinical data.

Subject	Age (y)	Sex	Weight (kg)	Creatinine clearance ($mL.h^{-1}.kg^{-1}$)	AsT ($iU.L^{-1}$)	AP ($iU.L^{-1}$)	Bilirubin ($\mu mol.L^{-1}$)	Albumin ($g.L^{-1}$)	AT_{III} (%)	Concomittant medication
Liver cirrhosis										
1	52	f	71	107	84	170	22	46	62	-
2	42	f	65	77	29	123	15	31	69	phytomenadion
3	23	m	84	71	450	108	28	23	81	-
4	44	f	55	73	110	400	120	24	86	hydrotalcite
5	56	m	82	88	71	73	12	31	45	oral iron, phytomenadion, hydrotalcite
6	19	f	63	113	126	148	15	30	60	azathioprine, phytomenadion
7	29	m	68	79	69	187	26	35	73	phytomenadion
Mean	38		69	87	134	172	34	31	68	
SD			(10)	(17)	(143)	(107)	(38)	(8)	(14)	
Chronic renal failure										
8	41	m	62	15	18	152	10	42	155	furosemide, oral, iron, vitamin D, allopurinol, captopril, diazepam
9	73	m	69	12	12	84	8	32	81	aluminium hydroxide, vitamin D, ranitidine
10	68	m	72	5	18	139	10	34	110	furosemide, isosorbidedinitrate, aluminium hydroxide
11	67	f	61	40	8	105	10	44	106	insulin
12	55	m	63	48	30	280	14	25	97	insulin, digoxin, oral iron
13	58	f	76	39	8	111	13	34	140	-
14	57	f	64	19	11	53	9	36	113	prednison, cyclofosfamid, ranitidine, aluminium hydroxide, dipyridamol
Mean	60		67	25	15	132	11	35	115	
SD			(6)	(17)	(8)	(73)	(2)	(6)	(25)	
Healthy subjects										
Mean	23		68	113	14	80	9	43	109	
SD			(8)	(14)	(86)	(28)	(3)	(4)	(13)	

AsT: aspartate aminotransferase; AP: alkaline phosphatase; AT_{III}: anti-thrombin III.

tase (AP, range 13-150 iU.L^{-1}). Patient 12 had an increased concentration of AP (280 iU.L^{-1}), possibly due to fatty liver. Some of the patients with renal disease had proteinuria, causing decreased concentrations of serum albumin.

Although the patients used various types of drugs, such as vitamins, iron salts and phosphate binders, they used no drugs known to interact with theophylline. None of the patients suffered from cardiac failure. Patient 9 and 12 were smokers (both patients 5-10 cigarettes per day).

The healthy control subjects ranged in age from 21-27 years (mean 23 years). All the healthy subjects underwent clinical evaluation, and their liver and renal function tests were in the normal range. They were all non-smokers. The study was approved by the Hospital's Medical Ethics Committee and all the subjects gave their informed consent to take part.

Study design

The patients were studied during their stay in hospital. And the healthy subjects stayed in a special ward. The patients and control subjects had their diets in the hospital. Some of the patients had a salt-restricted diet, and some renal patients had a protein-restricted diet. Xanthine-containing beverages were not allowed during the study.

On separate days the patients received a single intravenous infusion (over 30 min) of theophylline (5 mg.kg^{-1}) or enprofylline (0.75 mg.kg^{-1}). The dosage of enprofylline for the control subjects was 1 mg.kg^{-1}. The study days were separated by 3 to 7 days. Blood was sampled via an indwelling intravenous catheter in the arm opposite to the infusion arm. Blood samples were taken at 0,0.5,1,2,4, 6,8,14,24, 28, and 32 h after the start of the infusion.

Urine was collected for 24 h for the measurement of creatinine clearance. Heparinized blood samples were centrifuged and the plasma was separated and stored at –20° C until analysis. The analysis of plasma theophylline and enprofylline was carried out by high-performance liquid chromatography[13,14].

Pharmacokinetic calculations

The area under the plasma concentration-time curves (AUC) was determined using the linear trapezoid rule. The AUC from the last measured point (C_n) to infinity was estimated by C_n/K_{el}, where K_{el} is the elimination constant. K_{el} was calculated from the slope of the linear last part of the $\ln(C_n)$-time curve. Plasma half-life ($t_{1/2}$) was calculated as $(\ln 2)/K_{el}$. The apparent volume of distribution during steady state (V_{ss}) was calculated using the non-compartmental method of Benet and Galeazzi[15] and Smith et al.[16]. Total body clearance of the drug (CL) was calculated as dose/AUC.

Renal function and liver function tests

Renal function was assessed by creatinine clearance. Liver function was assessed by routine liver function tests.

The predictive value of renal function and liver function tests for the total body clearance of enprofylline and theophylline respectively was evaluated by calculating correlation coefficients between creatinine clearance or the liver function tests, and the measured total body clearance of enprofylline and theophylline respectively. In addition to seeking

correlations between liver function tests and theophylline clearance, we used the "population average" model of Jusko et al.[17] to find a predicted value for theophylline clearance in our subjects. These authors measured theophylline clearance in 200 patients and healthy subjects and assessed the influence of factors such as age, smoking, and liver disease on theophylline clearance.

Statistical analysis

Differences between and within groups were assessed by analysis of variance. P-values were calculated for a two-tailed probability.

Results

The mean values for the pharmacokinetics of theophylline and enprofylline in patients with liver cirrhosis, patients with chronic renal failure and the control group are summarized in Table 2.

Table 2. Theophylline and enprofylline pharmacokinetics in patients with liver cirrhosis, patients with chronic renal failure and healthy subjects.

	Patients with liver cirrhosis (n=7)	Patients with renal failure (n=7)	Healthy subjects (n=10)
Theophylline			
V_{ss} (L.kg^{-1})	0.50 (0.06)	0.43 (0.09)	0.48 (0.05)
K_{el} (h^{-1})	0.05 ** (0.02)	0.10 (0.04)	0.09 (0.02)
$t_{1/2}$ (h)	17.0 *** (6.8)	7.7 (2.1)	7.9 (2.2)
CL (mL.h^{-1}.kg^{-1})	24.4 * (11.8)	46.9 (20.9)	46.0 (11.0)
Enprofylline			
V_{ss} (L.kg^{-1})	0.60 (0.07)	0.51 (0.13)	0.57 (0.05)
K_{el} (h^{-1})	0.36 (0.12)	0.12 *** (0.07)	0.46 (0.07)
$t_{1/2}$ (h)	2.1 (0.7)	8.0 *** (4.7)	1.5 (0.2)
CL (mL.h^{-1}.kg^{-1})	250 (99)	64 *** (37)	289 (41)

V_{ss}: Volume of distribution during steady state. K_{el}: Elimination constant. $t_{1/2}$: time of half-life. CL: drug total body clearance. Results are presented as mean (SD). Significant group differences (anova): *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

Theophylline pharmacokinetics

V_{ss} did not differ significantly in the three groups. Theophylline $t_{1/2}$ was significantly increased in the patients with liver cirrhosis, compared with the other two groups ($p<0.01$). The range of $t_{1/2}$ was 7.1 to 27.5 h in the patients with liver cirrhosis, 3.5 to 10.4 h in the patients with renal disease and 6.0 to 12.0 h in the control group. Theophylline clearance was significantly decreased in the patients with liver disease, compared with both the other groups ($p<0.05$). The range of clearances was 13 to 47 $mL.h^{-1}.kg^{-1}$ in the liver patients, 20 to 87 $mL.h^{-1}.kg^{-1}$ in the renal patients and 32 to 60 $mL.h^{-1}.kg^{-1}$ in the healthy subjects.

Enprofylline pharmacokinetics

V_{ss} was not significantly different in the three groups. In the patients with renal disease enprofylline $t_{1/2}$ was significantly increased ($p<0.05$) and clearance significantly decreased ($p<0.001$), compared with the other two groups. The range of $t_{1/2}$ was 3.1 to 15.8 h in the renal patient group, 1.4 to 2.9 h in the liver patient group and 1.2 to 1.9 h in the controls. The range of clearance was 25 to 118 $mL.h^{-1}.kg^{-1}$ in the renal patient group,116 to 360 $mL.h^{-1}.kg^{-1}$ in the liver patient group and 225 to 353 $mL.h^{-1}.kg^{-1}$ in the healthy subjects.

Correlation between liver and renal function tests and xanthine pharmacokinetics

There was a significant correlation between some liver function tests and the clearance of theophylline. The correlation coefficients were, for serum AsT: $r=0.47$ ($p<0.05$), for serum AP: $r=0.41$ ($p<0.05$), for serum bilirubine $r=0.42$ ($p<0.05$). No significant linear correlation between serum albumin and AT_{III} concentrations and theophylline clearance was found. There was a significant linear correlation (Fig 1) between the predicted theophylline CL value, calculated by the method of Jusko[17] and the measured theophylline clearance ($r=0.50$, $p<0.05$, standard error of estimate: 15 $mL.h^{-1}.kg^{-1}$).

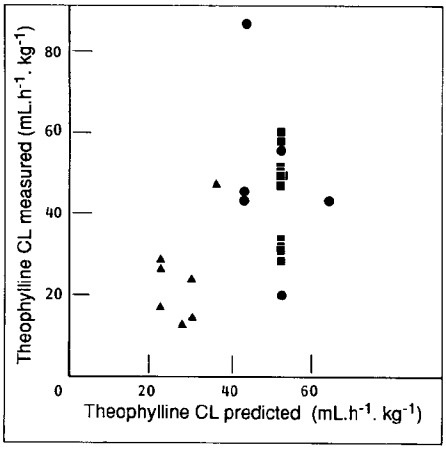

Figure 1. Correlation between predicted values of theophylline clearance and measured theophylline clearance.
▲: liver patients. ●: renal patients. ■: healthy subjects. The predicted value of theophylline clearance was calculated using the method of Jusko et al.[17]. A significant linear correlation was found ($r=0.50$, $p<0.05$, standard error of estimate 15 $mL.h^{-1}.kg^{-1}$)).

There was a significant linear correlation between creatinine clearance and enprofylline clearance. ($r=0.84$, $p<0.001$; the standard error of estimate was 62 $mL.h^{-1}.kg^{-1}$; fig.2).

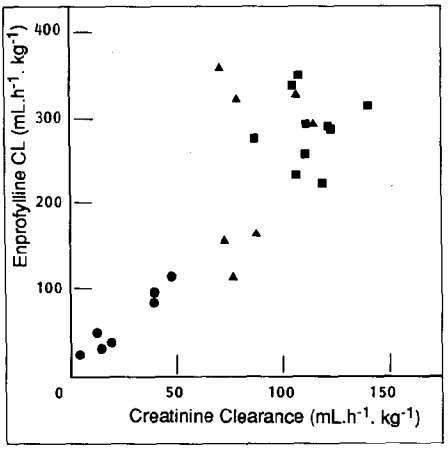

Figure 2. Correlation between creatinine clearance and enprofylline clearance. ▲: liver patients. ●: renal patients. ■: healthy subjects. There is a significant linear correlation ($r=0.85$, $p<0.001$; standard error of estimate 62 mL.h^{-1}.kg^{-1}).

Discussion

In this study we found, that the elimination rate of theophylline in the patients with liver cirrhosis and the other two groups (patients with renal disease and healthy subjects) differed by about a factor of two.

Enprofylline clearance was not decreased in patients with liver cirrhosis. The enprofylline plasma half-life values in the patients with liver cirrhosis are in contrast with the extremely prolonged plasma half-life of theophylline in these patients. On the other hand, the elimination rate of enprofylline in the patient group with renal disease was about four times smaller than in the patients with liver cirrhosis and the healthy controls. Theophylline clearance was not decreased in patients with renal disease. Apart from this, there was also a large interindividual variability in the clearances of both drugs separately within the groups.

The fall in theophylline elimination that we found in the patients with liver cirrhosis is in agreement with the results of several other studies[5,6,7,8,17]. Theophylline clearance can be more severely decreased in ascitic patients with decompensated liver cirrhosis. We did not include such patients in our study, because in these cases renal function is often also impaired as part of a hepato-renal syndrome.

In patients with renal disease the mean theophylline clearance for the group did not differ from the healthy subjects, although large inter-individual differences were found. We found (sometimes markedly) reduced enprofylline clearance values in these patients. This, together with the finding that enprofylline clearance is undisturbed in patients with liver cirrhosis, but with a normal renal function, is far from unexpected. It has been shown in several studies that enprofylline is mainly excreted unmetabolised by the kidney[10,11]. Borgå[10] et al. found that about 90% of an i.v. dose was excreted in the urine in healthy subjects. The values for enprofylline clearance that have been found by others (200-400 mL min^{-1} in healthy subjects), and in our study, indicate that tubular secretion plays an important role in the renal elimination of enprofylline, which has been recently confirmed[18]. In

patients with renal disease the part of enprofylline excretion that occurs extrarenally, increases (to about 20%). With increasing age the part of extra-renally excreted enprofylline also increases, partly due to factors other than the age-related decrease in creatinine clearance.

As was reported in previous studies we found a significant correlation between some of the liver function tests and theophylline clearance. However, the predictive value of these tests is poor. Moreover, in diseases such ase acute hepatitis and cholestasis, the predictive value of routine liver function tests may be even less[19]. The method described by Jusko et al.[17], using patient characteristics like disease state, age, smoking habit etc., was only slightly better in predicting theophylline clearance. The 95% confidence interval of the correlation calculated with this method was ± 31 mL.h^{-1}.kg^{-1} which implies that, although an estimate can be given of the mean theophylline clearance in patient groups using this method, there remains a rather large unexplained variance between individuals. Of course, with more elaborate liver function tests, such as like the antipyrine clearance test, a better correlation can be obtained with theophylline clearance. However, these tests are not very practical for routine clinical use. As has been reported previously by Lunell et al.[11,12] we have found a significant correlation between creatinine clearance and enprofylline clearance in the three studied groups. The 95% confidence interval of the correlation was ± 124 mL.h^{-1}.kg^{-1}, which implies that there is a considerable variation of enprofylline clearance, which cannot be explained by variations in creatinine clearance.

By comparing the estimated 95% confidence intervals of the predicted theophylline and enprofylline clearances, it can be seen, taking into account the order of magnitude of clearances of both drugs, that enprofylline clearance can be better predicted from a routinely available clinical laboratory test, than theophylline clearance from liver function tests or from the Jusko prediction model

Both theophylline and enprofylline clearances decrease with increasing age. Our patients and healthy subject groups were not entirely age-matched, and some bias may occur when results between subject groups are compared. This fact can only minimally have disturbed the comparison of the 2 predictive models, because in the Jusko model age is one of the independent variables, and the age-dependent variability of enprofylline is partly caused by the age-related change in creatinine clearance.

In conclusion, the present study has shown, that in patients with liver disease and severely impaired theophylline elimination, the clearance of enprofylline is undisturbed, when renal function is normal. Furthermore, in the entire group of liver patients with liver disease, renal disease and healthy control subjects creatinine clearance is of better predictive value for enprofylline clearance, than any of the used models for theophylline clearance. However, for both drugs there remains a rather large residual interindividual variance clearance, and it seems therefore to be a cautious policy, to monitor plasma concentrations at frequent intervals during maintenance treatment with both theophylline and enprofylline. Apart from this, in patients with severely disturbed liver or renal function, one should, in our opinion, choose for treatment with the drug that has the shortest (expected) plasma $t_{1/2}$.

References

1. Mitenko PA, Ogilvie RI (1973). Rational intravenous doses of theophylline. N Engl J Med 289:600-3.
2. Tang-Liu DD, Williams RL, Riegelman S (1982). Nonlinear theophylline elimination. Clin Pharmacol Ther 31:358-69.
3. Jonkman JHG, Upton RA (1984). Pharmacokinetic drug interactions with theophylline. Clinical Pharmacokinetics 9:309-34.
4. Piafsky KM, Sitar DS, Rangho RE, Ogilvie RI (1977). Theophylline kinetics in acute pulmonary oedema. Clin Pharmacol Ther 21:310-16.
5. Piafsky KM, Sitar DS, Rangho RE, Ogilvie RI (1977). Theophylline disposition in patients with hepatic cirrhosis. N Engl J Med 296:1495-7.
6. Mangione A, Imhof TE, Lee RV, Shum LY, Jusko WJ (1978). Pharmacokinetics of theophylline in hepatic disease. Chest 73:616-22.
7. Williams RL (1983). Drug administration in hepatic disease. (Drug therapy, ed. Koch-Weser J). N Engl J Med 309: 1616-22.
8. Jenne JW. (1986). Effect of disease state on theophylline elimination. J All Clin Immunol. 78: 727-35.
9. Persson CGA,Kjellin G (1981). Enprofylline,a principally new antiasthmatic xanthine. Acta Pharmacol Tox 49:317-20.
10. Borgå O,Anderson KE,Edholm LE, Fagerström PO, Lunell E,Persson CGA (1983). Pharmacokinetics of enprofylline in healthy subjects after intravenous single dose administration.Clin Pharmacol Ther 34:799-804.
11. Lunell E,Borgå O,Larsson R (1984). Pharmacokinetics of enprofylline in patients with impaired renal function after a single dose intravenous dose. Eur J Clin Pharmacol 26: 87-93.
12. Lunell E, Borgå, O (1987). Pharmacokinetics of enprofylline in elderly subjects. Eur J Clin Pharmacol 32: 67-70.
13. Jonkman JHG, Schoenmaker R, Greving JE, Zeeuw RA de (1980). Rapid and selective theophylline serum and saliva assay by means of high pressure liquid chromatography. Pharm Weekblad (Sci Ed) 2:49-53.
14. Edholm LE, Bodenas C, Heintz L, Ogren L (1985). Column liquid chromatography for the quantitive analysis of enprofylline in biological samples. In: Andersson KE and Persson CGA eds., Anti-asthma xanthines and adenosine, Amsterdam, Excerpta Medica 238-42.
15. Benet LZ, Galeazzi RL (1979). Non-compartmental determination of the steady state volume of distribution. J Pharma Sci 68:1071-4.
16. Smith DE, Gambertoglio JG, Vinecenti F, Benet LZ (1981). Furosemide pharmacokinetics and dynamics after kidney transplant. Clin Pharmacol Ther 30:105-13.
17. Jusko WJ, Gardner MJ, Mangione A, Schentag JJ, Koup JR, Vance JW (1979). Factors affecting theophylline clearances: Age, tobacco, marijuana, cirrhosis, congestive heart failure, obesity, oral contraceptives, benzodiazepines, barbiturates and ethanol. J Pharm Sci 68: 1358–66.
18. Borgå O, Larsson R, Lunell E (1986). Effects of probenicid on enprofylline kinetics in man Eur J Clin Pharmacol 30: 221-3.
19. Staib AH, Schuppan D, Lissner R, Zilly W, von Barnhard G, Richter E (1980). Pharmacokinetics and metabolism of theophylline in patients with liver diseases. Int J Clin Pharmacol Ther Toxicol 18: 500-2.

CHAPTER 6

CREATION OF FOUR CONSECUTIVE INSTANTANEOUSLY STEADY-STATE PLASMA CONCENTRATION PLATEAUS OF THEOPHYLLINE AND ENPROFYLLINE BY REPEATED INFUSIONS WITH EXPONENTIALLY DECREASING DELIVERY RATES

J.Kraan[1], L.Borgström[4], G.H.Koëter[1], M.Laseur[2], J.H.G.Jonkman[3], and O.E. de Noord[3]

[1]Department of Pulmonology, [2]Department of Pharmacy, University of Groningen, [3]Pharma Bio-Research International BV, Assen, the Netherlands, and [4]Research and Development Department, Pharmacokinetics Laboratory AB Draco, Lund, Sweden.

Summary

Repeated exponentially decreasing infusions have been used to administer theophylline and enprofylline to show wether it would be feasible to create consecutive plasma concentration plateaus within a few ours. The infusions were carried out on two separate study days in 8 stable asthmatic subjects. Before the infusion experiments, the pharmacokinetics of the substances in the individual subjects were determined on a separate day.

Plasma concentration rose to the desired level within 5 minutes after the start of the infusion at each dose level and a stable plasma concentration plateau was maintained during the following 90 minutes of infusion. It was possible to achieve 4 subsequent concentration plateaus within a 6 h period. Use of this infusion method resulted in predictable plasma concentrations at all levels and so the method appears safe when the required plasma concentrations are below the toxic level. Apart from clinical situations where effective doses of drugs must be administered rapidly the method appears to be useful in pharmacological dose-response studies.

Introduction

To study the dose-response relationship of, for example, bronchodilating drugs, intravenous infusion is the preferred route of administration because a stable plasma concentration of the drugs studied is required for a reliable interpretation of the dose-response data. In addition, as effects are to be examined at succeedingly increasing plasma concentra-

Eur J Clin Pharmacol 1988; 35:657-61

tions within a relatively short time period, it is desirable that the plasma concentrations plateaus are be reached within a few minutes.

For drugs whose distribution and elimination can be described by a simple one-compartment model, a stable plasma level can be attained instantaneously by administering an intravenous bolus dose, that raises the plasma concentration to the desired level, followed by an infusion given at the same rate as the elimination rate. For drugs that distribute also to a more peripheral or deeper compartment the situation is more complex[1-3]. The regimen described above would lead to a more or less prominent overshoot in plasma concentration, and the time to attain the stable plasma concentration would depend on the late elimination half-life. For drugs that obey two-compartment pharmacokinetics, a plateau plasma level can be attained instantaneously if the initial bolus dose that raises the concentration in the central compartment to the desired level is followed by an infusion that compensates for both the distribution to the peripheral compartment and elimination. To this end an infusion that decreases exponentially to a stable rate should be administered after the initial bolus dose[3-9]. This can be achieved by a micropressor controlled infusion system as described by Schwilden et al.[5,6]. Other authors have described a two bottle infusion system, in which a dilute solution of the drug is infused into a sealed rigid mixing bottle which contains a more concentrated solution of the drug[7,8,9].

We used the two-bottle infusion method in a study of the bronchodilatory and bronchoprotective properties of two xanthines, theophylline and enprofylline. Enprofylline is a recently developed xanthine with a strong bronchodilatory potency[10]. The drug is mainly excreted unmetabolized by the kidney, and is generally eliminated much more rapidly from the body than theophylline[11].

Eight stable asthmatic patients participated in the study. After their individual pharmacokinetic parameters for theophylline and enprofylline had been determined on a separate day, the drugs were infused on two other days. The pharmacokinetic part of the study was carried out to investigate the feasibility of creating four, consecutive, increasing plasma plateaus, at desired levels, within a period of 6 h, with 2 drugs with different elimination rates.

Subjects and methods

The study protocol was approved by the Hospitals Medical Ethics Committee. The study was performed in accordance with the Declaration of Helsinki.

Subjects

Eight asthmatic subjects gave their informed consent to participation in the study. Their age ranged from 23 to 34 years and their weight from 70 to 100 kg. When the subjects took theophylline as a maintenance drug they were instructed to withhold it for at least 3 days before each study day.

Pharmacokinetic characterization

The study took place on three separate days. On study day 1 the subjects were given a simultaneous intravenous infusion of theophylline and enprofylline. The doses of theo-

phylline and enprofylline were 2mg.kg^{-1} and 1 mg.kg^{-1} respectively. The drugs were infused slowly at a constant rate for ten minutes via an antecubital vein, with the subject recumbant. From an antecubital vein on the contralateral side blood was sampled at 10, 15, 20, 25, 30, 40 min and 1, 2, 3, 4, 5, 6, and 7 h. after the start of the infusion. The plasma concentrations of theophylline and enprofylline were determined by high pressure liquid chromatography[12,13].

For each subject and drug a two-compartment model $C = A.e^{-\alpha} + B.e^{-\beta}$, where A, B, α, and β have their conventional meanings, was fitted to the data with help of a non-linear curve fitting program, Maxfit[14]. Based on the individual values of the coefficients and constants the microscopic rate constants for transfer of drug between the central and peripheral compartments and V_{ss} and V_c were calculated. V_{ss} is the volume of distribution at steady state[15] and V_c is the volume of the central compartment. The obtained pharmacokinetic parameters were used to calculate an individualized dosing regime for each subject and xanthine as described by Riddell et al.[8]. The interval between study day 1 and the other part of the study was 6 weeks at the most.

Infusion technique

The infusion regime used on study days 2 and 3 was designed to produce four, consecutive, stable plasma concentration levels either of theophylline or enprofylline. The intended plasma concentrations were 5, 10, 15 and 20 mg.L^{-1} for theophylline and 1.25, 2.5, 3.75 and 5 mg.L^{-1} for enprofylline. The infusion system (Figure 1) consisted of a large vial a reservoir, with a low drug concentration, and a small vial the mixing chamber, with a high drug concentration. The vials were interconnected with each other via plastic

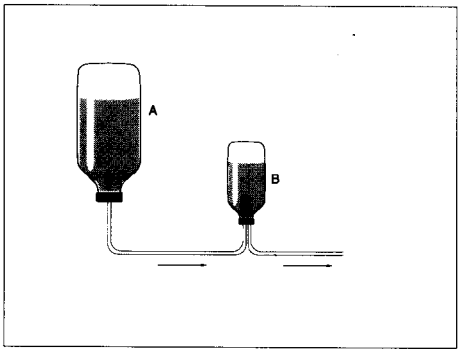

Figure 1. Infusion system. During infusion solution is removed from the small mixing chamber (B), and replaced by solution coming from the large reservoir vial (A). For further explanation see text (infusion technique).

tubings and the infusions were done with a rate-controlled infusion pump. During the infusion, solution was removed at a constant rate from the the mixing chamber, and replaced at the same rate by solution from the reservoir vial. Thus the volume of solute, in the mixing chamber, was kept constant during the infusion, while the concentration of drug in the mixing chamber decreased exponentially with time.

On study days 2 and 3 an infusion was given over a total period of 360 min. First an intravenous bolus dose was given during 3 min to raise the xanthine concentration in the central compartment to the first plateau level. The bolus dose was immediately followed by an exponentially decreasing infusion during the following 87 min. To reach the four

successive concentration plateaus, the procedure was repeated at 90, 180, and 270 min. The initial drug concentrations in the reservoir vial and the mixing chamber were calculated separately for each substance, step, and patient by applying the equations given by Riddell et al.[8]. As the rate of delivery of solvent was kept constant at 1 ml.min^{-1}, a new reservoir vial and mixing chamber had to be used for each dose step. Blood for xanthine analysis was sampled 5, 55 and 85 min after the start of each bolus infusion, in all providing 12 samples from each experiment.

Results

The individual and mean values of the constants and coefficients after the pharmacokinetic curve fitting, as well as the calculated values for V_c, V_{ss} and $t_{1/2}$, are given in Tables 1 and 2. The plasma concentrations during the stepwise infusion of theophylline and enpro-

Table 1. Theohylline: pharmacokinetic constants and coefficients obtained from the model study on day 1, and the calculated values for V_c, V_{ss}, and $t_{1/2}$.

Subj.	A (mg.L^{-1})	α (min^{-1})	B (mg.L^{-1})	β (min^{-1})	V_c (L.kg^{-1})	V_{ss} (L.kg^{-1})	$t_{1/2}$ (h)
1	29.5	0.180	4.04	0.00145	0.07	0.52	7.7
2	7.8	0.101	5.02	0.00241	0.16	0.38	4.8
3	17.3	0.145	3.61	0.00186	0.10	0.53	6.1
4	27.3	0.165	4.53	0.00178	0.07	0.44	6.4
5	12.4	0.147	3.99	0.00276	0.13	0.48	4.1
6	18.6	0.151	4.30	0.00211	0.09	0.46	5.5
7	17.8	0.160	4.74	0.00216	0.09	0.38	5.3
8	21.0	0.153	4.57	0.00136	0.09	0.45	8.3
Mean	19.0	0.150	4.35	0.00199	0.10	0.46	6.0
SEM	2.5	0.008	0.16	0.00017	0.01	0.02	0.5

Table 2. Enprofylline: pharmacokinetic constants and coefficients obtained from the model study on day 1, and the calculated values for V_c, V_{ss}, and $t_{1/2}$.

Subj.	A (mg.L^{-1})	α (min^{-1})	B (mg.L^{-1})	β (min^{-1})	V_c (L.kg^{-1})	V_{ss} (L.kg^{-1})	$t_{1/2}$ (h)
1	8.60	0.127	1.38	0.00673	0.13	0.55	1.7
2	5.35	0.095	1.70	0.00726	0.17	0.47	1.6
3	7.60	0.143	1.42	0.00756	0.14	0.54	1.5
4	8.70	0.117	1.42	0.00484	0.12	0.55	2.4
5	6.64	0.120	1.43	0.00587	0.16	0.59	2.0
6	11.8	0.167	1.52	0.00760	0.11	0.54	1.5
7	5.89	0.104	1.73	0.00548	0.14	0.44	2.1
8	16.2	0.190	1.81	0.00734	0.09	0.50	1.6
Mean	8.85	0.133	1.55	0.00659	0.13	0.52	1.8
SEM	(1.27)	(0.011)	(0.06)	(0.00037)	(0.01)	(0.02)	(0.1)

Table 3. Observed plasma concentrations of theophylline after stepwise infusion aimed at 5, 10, 15, and 20 mg.L^{-1}.

	Level 1			Level 2			Level 3			Level 4		
Subj.	5'	55'	85'	5'	55'	85'	5'	55'	85'	5'	55'	85'
1	4.0	5.7	5.6	9.7	10.4	10.5	12.9	16.5	16.5	-	-	-
2	7.2	5.5	5.3	13.2	11.0	10.5	14.7	15.6	15.3	24.5	22.0	21.4
3	4.9	5.1	4.9	8.0	10.8	10.4	13.8	14.7	15.5	-	-	-
4	5.1	5.7	5.1	10.3	10.1	10.2	15.0	14.2	14.6	20.5	21.6	22.3
5	6.6	5.3	5.2	11.5	10.7	10.4	15.2	15.9	15.7	-	-	-
6	3.7	5.0	4.9	7.7	9.2	9.5	13.0	14.0	14.2	19.6	20.4	20.6
7	5.5	4.5	4.5	8.3	9.0	9.1	15.0	13.2	13.7	-	-	-
8	6.0	5.3	5.3	10.8	10.4	9.8	12.9	15.6	14.9	17.7	20.2	20.5
Mean	5.4	5.3	5.1	9.9	10.2	10.1	14.1	15.0	15.1	20.6	21.1	21.2
SEM	0.4	0.1	0.1	0.7	0.3	0.2	0.4	0.4	0.3	1.4	0.4	0.4

Experimental time is time within each plateau.

Table 4. Observed plasma concentrations of enprofylline after stepwise infusion aimed at 1.25, 2.5, 3.75 and 5 mg.L^{-1}.

	Level 1			Level 2			Level 3			Level 4		
Subj.	5'	55'	85'	5'	55'	85'	5'	55'	85'	5'	55'	85'
1	1.2	1.3	1.3	2.6	2.6	2.7	3.6	4.1	4.5	5.7	5.8	-
2	2.9	1.5	1.6	3.0	3.1	3.2	5.1	5.1	5.2	5.7	6.0	6.0
3	1.4	1.3	1.3	2.3	2.8	2.8	3.6	4.2	4.4	-	-	-
4	1.4	1.4	1.3	2.8	2.6	2.5	4.2	3.9	3.7	5.5	5.1	5.4
5	1.4	1.1	1.2	2.9	2.4	2.3	3.4	3.5	3.4	-	-	-
6	0.9	1.0	1.1	2.1	2.3	2.5	3.8	4.3	4.2	5.3	6.0	6.3
7	1.4	1.1	-	2.3	2.3	2.4	3.3	3.6	3.0	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-
Mean	1.5	1.2	1.3	2.6	2.6	2.6	3.9	4.1	4.1	5.6	5.7	5.9
SEM	(0.2)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.2)	(0.2)	(0.3)	(0.1)	(0.2)	(0.3)

Experimental time is time within each plateau.

fylline are shown in Tables 3 and 4, and the mean values are given in Fig. 2 and 3. Due to an error in calculating the dose regimen for enprofylline in subject 8, the plasma concentrations after the stepwise infusions of enprofylline in him did not remain stable. Those data have been excluded from the calculations. Side effects as nausea, vomiting and headache occurred at the highest dosage step in subject 1,5 and 8. For this reason it was decided to omit the last dose step in the succeeding experiments.

For theophylline the desired levels were 5,10,15 and 20 mg.L^{-1} respectively. A deviation of more than 2.5 mg.L^{-1} was seen in 2 out of 28 occasions at 5 min but on no occasion at 55 and 85 min after the start of the dosage step.

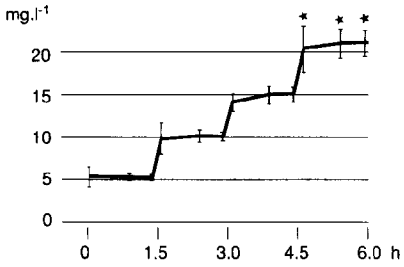

Figure 2. Plasma concentrations of theophylline during the stepwise infusion. Mean values (SEM) observed at 5, 55, and 85 min. after each bolus infusion are represented. See Table 3 for individual data. (*n=4).

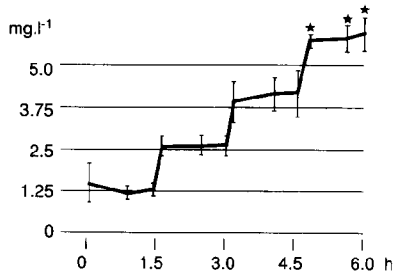

Figure 3. Plasma concentrations of enprofylline during the stepwise infusion. Mean values (SEM) observed at 5, 55, and 85 min. after each bolus infusion are represented. See Table 4 for individual data. (*n=4).

For enprofylline the desired levels were 1.25, 2.5, 3.75 and 5 mg.L^{-1} respectively. A deviation of more than 1 mg.L^{-1} was seen in 2 out of 25 occasions at 5 min and in 1 occasion at 55 and 85 min after the start of the dosage step.

Discussion

The infusion method used in the present study made it possible to create four instantaneous stable plasma concentrations of theophylline or enprofylline. The plasma drug concentrations reached 5 min after the initial bolus infusion, were usually very near the desired levels, and the concentrations after 55 and 85 min remained stable. To attain stepwise increases in the plasma concentration plateaus, the infusion procedure was repeated four times within the 6 h period. The plasma concentrations during the fourth dose step were as stable as those during the first dose step, which supports the validity of the method.

The results of the present study show that the method is safe, and that a desired plasma concentration can be instantaneously attained, without side effects when the desired concentrations are below the toxic level. This is of importance when immediate therapeutic effect is desired for example in treatment of severe bronchial obstruction. Use of the infusion method to obtain stepwise increases in plasma plateaus can be of importance in dose-response studies, as large initial fluctuations of drug concentrations may be a source of error in such experiments.

In the present study, for practical purposes, the pharmacokinetic parameters of theophylline and enprofylline were determined after a simultaneous bolus infusion of both drugs. It is not possible to exclude an interaction having some effect on the pharmacokinetic behaviour of the drugs. As the two drugs are eliminated by different routes (theo-

phylline by the hepatic, and enprofylline by the renal route) a pharmacokinetic interaction is not likely. This is supported by the fact that the pharmacokinetic values that were found did not deviate from those reported in literature[16,17], and the good fit of the experimental plasma concentrations in the infusion experiments to the desired levels shows the validity of the pharmacokinetic data obtained in this way.

In conclusion, the present results demonstrate that using infusions with exponentially decreasing delivery rates it makes it possible effectively to obtain instantaneous stable plasma drug levels. The method may be useful for treatment of disease states, in which rapid attainment of effective blood drug levels is required, without the hazard of potentially toxic peak concentrations. The infusion method may also be very useful in pharmacological dose-response studies.

References

1. Aps C, Bell JA, Jenkins BS, Poole-Wilson PA, Reynolds F (1976) Logical approach to lidocaine therapy Br Med J 1:13-5.
2. Wagner JG (1974). A safe method for rapidly achieving plasma concentration plateaus. Clin Pharmacol Ther 16: 691-700.
3. Zhang WG (1983). Dosage regimen calculation of "intravenous injection plus intravenous infusion" for two compartment drugs. Acta Pharmaceut Sin 18: 561-8.
4. Kruger-Thiemer E (1968) Continuous intravenous infusion and multi-compartment accumulation. Eur J Pharmacol 4:317-24.
5. Schwilden H. (1981) A general method for calculating the dosage scheme in linear pharmacokinetics. Eur J Clin Pharmacol 20: 379-86.
6. Lauven PM, Stoeckel H, Schwilden H. (1982) Ein pharmakokinetisch begrundetes Infusionsmodell fur Midazolam. Eine microprocessorgesteuerte Applikationsform zur Erreichung konstanter Plasmaspiegel. Anaesthesist; 31: 15-20.
7. Song JG, Sun RY (1983). Pharmacokinetics of double-bottle intravenous infusion. Acta Pharmaceut Sin 18:732-40.
8. Riddell JG, McAllister CB, Wilkinson GR, Wood AJJ, Roden DM (1984) A new method for constant plasma drug concentrations: application to lidocaine. Ann Int Med 100:25-8.
9. McMurray TJ, Robinson FP, Dundee JW, Riddell JG, McClean E. (1986) A method for producing constant plasma concentrations of drugs. Br J Anaesth 58:1085-90.
10. Persson CGA,Kjellin G. (1981) Enprofylline,a principally new anti-asthmatic xanthine. Acta Pharmacol Tox 49:317-20.
11. Borgå O,Anderson KE,Edholm LE, Fagerström PO, Lunell E,Persson CGA (1983) Pharmacokinetics of enprofylline in healthy subjects after intravenous single dose administration.Clin Pharmacol Ther 34:799-804.
12. Jonkman JHG, Schoenmaker R, Greving JE, Zeeuw RA de (1980) Rapid and selective theophylline serum and saliva assay by means of high pressure liquid chromatography. Pharm Weekblad (Sci Ed) 2:49-53.
13. Edholm LE, Bodenas C, Heintz L, Ogren L (1985) Column liquid chromatography for the quantitive analysis of enprofylline in biological samples. In: Andersson KE and Persson CGA eds., Anti-asthma xanthines and adenosine, Amsterdam, Excerpta Medica 238-42.

14. Maxfit. Nonlinear parameter estimation via likelyhood maximation. Users guide. Uppsala, Sweden (1985).

15. Benet LZ, Galeazzi RL (1979)Non-compartmental determination of the steady state volume of distribution. J Pharma Sci 68:1071-4.

16. Hendeles L, Weinberger M (1983) Drugs in perspective: Theophylline, a state of the art. Pharmacother 3: 2-44

17. Jonkman JHG, Borgå O, Boon WJV van der, Grasmeyer G, Ekman-Hakansson I. (1986) Pharmacokinetics of enprofylline: relationship to age, sex, smoking habits and creatinine clearance. In: Andersson KE and Persson CGA eds., Anti-asthma xanthines and adenosine, Amsterdam, Excerpta Medica: 248-54.

CHAPTER 7

THE EFFECT OF THEOPHYLLINE AND ENPROFYLLINE ON BRONCHIAL HYPERRESPONSIVENESS

G.H. Koëter[1], J. Kraan[1], M. Boorsma[3], J.H.G. Jonkman[2], Th.van der Mark[1]

Department of Pulmonology and Lung Function, University Hospital, Groningen (1), Pharma Bio Research, Assen (2), and Astra Pharmaceutica, Rijswijk (3), The Netherlands

Summary

The effect of increasing intravenous doses of theophylline and enprofylline (a new xanthine derivative) on bronchial responsiveness to methacholine was studied in 8 asthmatic patients. Methacholine provocations were carried out on three separate days before and after increasing doses of theophylline, enprofylline, and placebo, a double-blind study design being used. Methacholine responsiveness was determined as the provocation concentration causing a fall of 20% in FEV_1 (PC_{20}).

The patients were characterized pharmacokinetically before the main study to provide an individual dosage scheme for each patient that would provide rapid steady-state plasma concentration plateaus of 5, 10, and 15 $mg.L^{-1}$ for theophylline, and 1.25, 2.5, and 3.75 $mg.L^{-1}$ for enprofylline. Dose increments were given at 90 minute intervals. FEV_1 showed a small progressive decrease in FEV_1 following placebo; it remained high in relation to placebo after both drugs and this effect was dose related. Methacholine PC_{20} values decreased significantly following placebo; the PC_{20} values were significantly higher after theophylline and enprofylline treatment than after placebo (maximum difference 2.0 and 1.7 doubling doses of methacholine). The effect of both drugs was dose related. Thus theophylline and enprofylline when given intravenously cause a small dose related increase in FEV_1 and methacholine PC_{20} when compared with placebo.

Thorax 1989; 44:1022-6

Introduction

Theophylline is widely used as maintenance treatment for moderately severe asthma[1]. In addition to causing a dosage related bronchodilatation[2], theophylline provides some protection against the effects of constrictor agents such as histamine and methacholine[3-7]. Enprofylline a recently developed xanthine derivative, is 3 to 5 times more potent as a bronchodilator than theophylline[8,9], and might have advantages over theophylline, if central nervous system side effects are less[10].

In the present study increasing doses of enprofylline and theophylline were given intravenously to patients with asthma to examine the protective effect of theophylline and enprofylline on bronchoconstriction induced by inhaled methacholine and to relate this to the plasma concentrations of the 2 drugs. Before the study the pharmacokinetic parameters of both drugs were determined for each patient, so that individualised drug doses could be given and plasma concentrations held within narrow limits.

Methods

Patients

Eight male asthmatic patients (mean age 29 years, range 23 to 34 years) gave their written informed consent to participate in the study. The clinical characteristics of each patient are shown in table 1. Patients had to show an increase in forced expiratory volume in one

Table 1. Clinical data of the patients.

Pat. no	Age (y)	VC (L)	FEV_1 (L)	FEV_1 (%predicted)	%increase above initial FEV_1 after terbutaline	Methacholine PC_{20} ($mg.mL^{-1}$)
1	34	5.40	3.25	71	18	0.78
2	29	5.50	3.00	66	40	0.10
3	24	5.50	3.40	71	25	0.24
4	32	4.90	2.10	53	18	0.10
5	23	5.40	3.60	71	19	1.07
6	29	5.30	3.25	77	21	1.41
7	25	6.30	4.30	89	16	0.18
8	33	3.70	2.20	62	37	0.03
Mean	29	5.25	3.14	70	24	0.24*
SD		0.74	0.72	11	9	

The terbutaline effect was tested on a separate study day.The inhaled dose was 0.500 mg (2 puffs).
*: Geometric mean value.

second (FEV_1) of at least 15% after 200 μg terbutaline. All patients had increased bronchial responsiveness to methacholine. (Provocation concentration causing a fall of 20% in FEV_1 (PC_{20}) below $4mg.L^{-1}$; see: pulmonary function and inhalation provocation tests).

Asthma was well controlled in all subjects by a small dose of an inhaled bronchodilator and/or a prophylactic drug (sodium cromoglycate or an inhaled corticosteroid). Two patients used theophylline as maintenance treatment. This drug was withheld for at least one week before each study day. No patient used oral steroids regularly.

Study design

Pharmacokinetic indices for each individual were measured on separate days after an intravenous bolus of 2 $mg.kg^{-1}$ theophylline and 1 $mg.kg^{-1}$ enprofylline. Total body clearance, volume of distribution at steady state, and volume of the central compartment were calculated from the drug plasma time-concentration curve.

The challenge tests were carried out on three days, before and after theophylline, enprofylline and placebo. After baseline lung function measurements an inhalation provocation test with methacholine was carried out. Baseline FEV_1 values on the 3 study days had to be within 10% of the mean value for the 3 days, and the initial methacholine PC_{20} values had to be within 1 dose step on the three days. After the baseline measurement an infusion was given over a total period of 270 min. An intravenous bolus dose was given initially over 3 min. to raise the plasma xanthine concentration to the first plateau level. The bolus dose was immediately followed by an exponentially decreasing dose by infusion during the succeeding 87 min. The procedure was repeated at 90, and 180 minutes in an attempt to achieve the three successive concentration plateaus with increasing doses of drug[11]. The plasma levels aimed for were 5.0, 10.0 and 15.0 $mg.L^{-1}$ for theophylline and 1.25, 2.50, and 3.75 $mg.L^{-1}$ for enprofylline. The drug doses needed to achieve these goals, were calculated from the pharmacokinetic measurements for each subject and prepared by the pharmacist in individual bottles for each subject to ensure that the study was blind for both the investigators and the patients.

The inhalation provocation with methacholine was repeated on three occasions with each drug 60 minutes after the start of each infusion at an increased drug concentration. The patients were not allowed to have beverages containing xanthine during the study days.

Pulmonary function and inhalation provocation test

Slow inspiratory vital capacity (VC) and FEV_1 were measured with a water-sealed spirometer. Increasing concentrations of methacholine
were inhaled from a "Wiesbadener doppelspray" (Wiesbadener Inhalator-Vertrieb, Wiesbaden, W. Germany) with an airflow 8 $L.min^{-1}$. The output of the nebulizer was 0.12 ± 0.02 $mL.min^{-1}$. The aerosols were inhaled during tidal breathing, with the patient wearing a nose clip. Doubling concentrations of methacholine from 0.032 to 8 $mg.mL^{-1}$ were inhaled for 2 minutes at 5 minutes intervals until the FEV_1 had fallen by 20% from baseline FEV_1.

Log dose methacholine was plotted against FEV_1 and the provocation concentration of methacholine required to produce a fall in FEV_1 of 20% was measured by interpolation (PC_{20})[12].

The theophylline and enprofylline concentrations in plasma were determined by using a high pressure liquid chromatography method[13,14].

Statistical analysis

Methacholine PC_{20} values were log transformed. The response to theophylline and enprofylline was expressed as the difference between log PC_{20} on the active treatment days and placebo days. Change in FEV_1 values was expressed as percentage (%) of the baseline value on each day. With these as dependent variables, analysis of variance was carried out with treatment and dosage step as independent variables[15], followed by Duncan's Multiple Range Test to establish differences between groups. To assess dose dependency, linear regression analysis was carried out, to relate plasma xanthine levels to changes in FEV_1 and methacholine PC_{20} (excluding baseline measurements) for both theophylline and enprofylline separately.

Results

Theophylline and enprofylline plasma concentrations

Plasma concentration remained stable during the continuous infusion period after each increment. The mean plasma concentrations (table 2) deviated little from the concentrations we aimed at (theophylline 5.3, 10.2, 15 $mg.L^{-1}$, enprofylline 1.2, 2.4, 3.7 $mg.L^{-1}$).

Table 2. Plasma xanthine concentration at methacholine PC_{20} measurement.

	Theophylline ($mg.L^{-1}$)			Enprofylline ($mg.L^{-1}$)		
	Dose step			Dose step		
Patient no	1	2	3	1	2	3
1	5.7	10.4	16.5	1.3	2.6	4.1
2	5.5	11.0	15.6	1.5	3.1	5.1
3	5.1	10.8	14.7	1.3	2.8	4.2
4	5.7	10.1	14.2	1.4	2.6	3.9
5	5.3	10.7	15.9	1.1	2.4	3.5
6	5.0	9.2	14.0	1.0	2.3	3.5
7	4.5	9.0	13.2	1.1	2.3	3.6
8	5.3	10.4	15.6	1.0	1.5	2.0
Mean	5.3	10.2	15.0	1.2	2.4	3.7
SD	0.4	0.7	1.1	0.2	0.5	0.9

Lung function and methacholine PC20: Effect of increasing theophylline and enprofylline concentrations

Mean baseline values of FEV_1 and methacholine PC_{20} on the three study days did not differ significantly (table 3). Mean change in FEV_1 and PC_{20} from baseline after increasing dosages of active drug and placebo are shown in figures 1 and 2.

Placebo

After placebo FEV_1 decreased gradually from 68% predicted before methacholine challenge to 58% before the fourth challenge (n.s.). Geometric mean methacholine PC_{20} decreased from 0.25 $mg.L^{-1}$ to 0.10 $mg.L^{-1}$ after the fourth challenge ($p<0.01$).

Theophylline and enprofylline

There was a dose related effect of both drugs on FEV_1 by comparison with the change after placebo (analysis of variance, $p<0.01$). There was a significant relationship between the increase in FEV_1 and the plasma concentrations of both drugs ($r=0.57$ for theophylline and $r=0.46$ for enprofylline; $p<0.01$).

The increase in FEV_1 achieved significance after the first dose increasement for theophylline ($p<0.05$) and after the second for enprofylline ($p<0.01$).

Methacholine PC_{20} differed progressively from placebo with increasing plasma concentrations of theophylline and enprofylline (analysis of variance, $p<0.05$), a significant difference being apparent at the lowest doses (theophylline 5.0 $mg.L^{-1}$, $p<0.01$; enprofylline 1.2 $mg.L^{-1}$, $p<0.05$). Change in methacholine PC_{20} was related to plasma drug concentration ($r=0.46$ for theophylline and 0.35 for enprofylline, $p<0.05$).

There were no significant differences between the effect of theophylline and enprofylline on FEV_1 and methacholine PC_{20}, at any of the three dose increments.

Side effects

Slight to moderate headache and nausea were noticed by three patients with the highest dose of theophylline and headache by three patients with the highest dose of enprofylline. One patient had slight headache during the placebo treatment day.

Table 3. Effect of increasing doses of theophylline and enprofylline on FEV_1 (Mean (SD)) and methacholine PC_{20} (geometric mean (geometric range of SEM)).

	Saline		Theophylline		Enprofylline	
Xanthine plasma level	FEV_1 (%pred)	PC_{20} ($mg.mL^{-1}$)	FEV_1 (%pred)	PC_{20} ($mg.mL^{-1}$)	FEV_1 (%pred)	PC_{20} ($mg.mL^{-1}$)
0	0.68 (0.04)	0.25 (0.15-0.40)	0.70 (0.04)	0.21 (0.13-0.33)	0.71 (0.05)	0.21 (0.15-0.30)
1	0.65 (0.04)	0.20 (0.14-0.31)	0.74 (0.03)	0.27 (0.19-0.39)	0.73 (0.04)	0.30 (0.21-0.44)
2	0.64 (0.04)	0.12 (0.07-0.21)	0.75 (0.03)	0.35 (0.23-0.51)	0.76 (0.04)	0.34 (0.22-0.52)
3	0.58 (0.04)	0.10 (0.06-0.17)	0.75 (0.03)	0.32 (0.23-0.46)	0.78 (0.05)	0.28 (0.19-0.41)

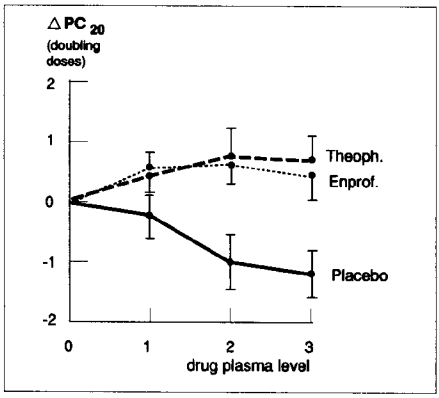

Figure 1. The effect of increasing theophylline and enprofylline plasma levels (compared with placebo) on FEV_1, expressed as the percentage change from baseline. The bars indicate ± SEM.
The drug plasma levels 1, 2, and 3 correspond with 5.3, 10.2, 15.0 $mg.L^{-1}$ for theophylline, and 1.2, 2.4, 3.7, $mg.L^{-1}$ for enprofylline.

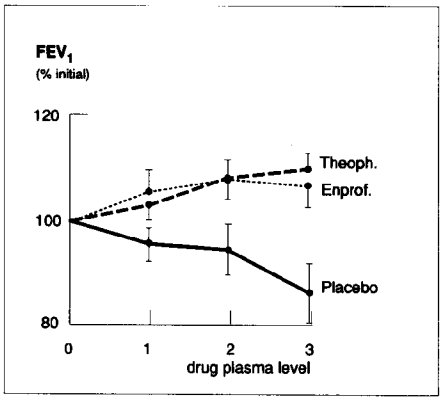

Figure 2. The effect of increasing theophylline and enprofylline plasma levels (compared with placebo) on PC_{20}, expressed as the change in doubling doses from baseline. The bars indicate ± geometric range of SEM.
The drug plasma levels 1, 2, and 3 correspond with 5.3, 10.2, 15.0 $mg.L^{-1}$ for theophylline, and 1.2, 2.4, 3.7, $mg.L^{-1}$ for enprofylline.

Discussion

There was a progressive decline in FEV_1 and methacholine PC_{20} after repeated methacholine challenges on the placebo day. The likely explanation for this decline is a cumulative bronchoconstrictor effect of methacholine, the interval between the challenges probably being too short for complete recovery[16]. Consequently the present study cannot determine the maximal achievable bronchodilation in these patients or the xanthine plasma concentration at which this occurs. Comparison of drug and placebo, however, in terms of change in FEV_1 and methacholine PC_{20} provides a measure of xanthine-induced protection, and the dosage dependency of this effect can be assessed. Theophylline and enprofylline caused a dose related improvement in FEV_1 and methacholine PC_{20} by comparison with placebo, significant differences being seen with relatively low plasma concentrations of both drugs.

Several studies have shown a moderate protective effect of theophylline against histamine[3-7], and methacholine[4,7] provocation. Few studies have looked at the effect of increasing doses of theophylline on bronchial hyperresponsiveness. Cockcroft et al.[3] found significant protection against histamine provocation when serum theophylline levels were above 10 $mg.L^{-1}$. In another study[7] no correlation was seen between the pro-

tective effect of theophylline on histamine challenges and theophylline plasma concentration, possibly because the comparison was between subjects and not within subjects. In a recent report Magnussen and coworkers[17] showed dose related protection of theophylline against histamine challenges and significant protection with low plasma theophylline concentrations (6 $mg.L^{-1}$), in accordance with our results. In our study the maximum protective effect of theophylline and enprofylline (that is, difference from placebo) was 2.0 doubling doses of methacholine for theophylline and 1.7 for enprofylline.

It can be concluded from our study that, although theophylline and enprofylline provide dose related protection against methacholine, the effect is relatively small. Both drugs provide protection at relatively low plasma concentrations. Increasing the theophylline plasma concentration from 10 $mg.L^{-1}$ to 15 $mg.L^{-1}$ and enprofylline concentration from 2.5 to 3.7 $mg.L^{-1}$ did not lead to an important further contribution to the protective effect. The results are in accordance with recent evidence that most of the possible bronchodilatory effect of theophylline is achieved at relatively low plasma concentrations[18,19].

References

1. Hendeles L, and Weinberger M. Theophylline: a State of the Art review. Pharmacotherapy 1983; 3:2-44
2. Mitenko, PA, and Ogilvie R.I. Rational intravenous doses of theophylline. N Engl J Med 1973; 289:600-3.
3. Cockcroft DW, Killian DN, Mellon JJH, Hargreave FE. Protective effect of drugs on histamine-induced asthma. Thorax 1977; 32:429-37.
4. Koëter GH, Meurs H, Jonkman JHG. De Vries K. Protective effect of choline theophyllinate on histamine, acethylcholine, and propranolol-induced airflow obstruction. Respiration 1984; 45:139-46.
5. Cartier A, Lemire I, L'Archeveque J, Ghezzo H, Martin R, and Malo JL. Theophylline partially inhibits bronchoconstriction caused by inhaled histamine in subjects with asthma. J Allergy Clin Immunology 1986; 77:570-75.
6. Levene S, and McKenzie SA. Protective effect of theophylline on histamine-induced bronchoconstriction in asthmatic children. Br J Clin Pharmac 1986; 21:445-9.
7. McWilliams BC, Menenokz R, Kelly WH, Howick J. Effects of theophylline on inhaled methacholine and histamine in asthmatic children. Am Rev Respir Dis 1984; 130:193-7.
8. Persson CGH, and Kjellin G. Enprofylline, a principally new antiasthmatic xanthine. Acta Pharmacol Toxicol 1981; 49:313-6.
9. Lunell E, Svedmyr N, Andersson KE, and Persson CGA. Effects of enprofylline, a xanthine lacking adenosine receptor antagonism, in patients with chronic obstructive lung disease. Eur J Clin Pharmacol 1982: 22:395-402.
10. Persson CGA, Erjefalt I, Edholm E, Karlsson JA, Lamm CJ. Tracheal relaxant and cardiostimulant effects of xanthines can be differentiated from diuretic and CNS-stimulant actions of xanthines. Role of adenosine antagonism. Life Sci 1982; 31:2673-81.
11. Kraan J, Borgström L, Koëter GH, Laseur M, De Noord OE, Jonkman JHG. Creation of four consecutive instanteneous steady state plasma concentration plateaus of theophylline and enprofylline by repeated infusions with exponentially decreasing delivery rates. Eur J Clin Pharmacol 1988; 35:657-61.

12. Kraan J, Koeter GH, v.d.Mark ThW, Sluiter HJ, and de Vries K. Changes in bronchial hyperreactivity induced by 4 weeks of treatment with antiasthmatic drugs in patients with allergic asthma. J Allergy Clin Immunology 1985; 76:628-36.

13. Jonkman JHG, Schoenmaker R, Greving JE, De Zeeuw RA. Rapid and selective theophylline and saliva assay by means of high pressure liquid chromatography. Pharm Weekblad (Sci ed.) 1980; 2:49-53.

14. Edholm LE, Bodenas C, Heintz L, Ogren L. Column liquid chromatography for the quantitative analysis of enprofylline in biological samples. In: Andersson KE and Persson CGA, eds., Anti- asthma xanthines and adenosine, Amsterdam, Excerpta Medica 1985; 238-42.

15. Nie NH, Hull Ch, Jenkins JG, Steinbrecher K, Bent DH. SPSS handbook, releases 7 to 9, Mcgraw-Hill, New York 1981

16. Cartier A, Malo JL, Begin P, Sestier M, Martin RR. Time course of the bronchoconstriction induced by inhaled histamine and methacholine. J Appl Physiol 1983; 54:821-6.

17. Magnussen H, Reuss G, Jorres R. Theophylline has a dose-related effect on the airway response to inhaled histamine and methacholine in asthmatics. Am Rev Respir Dis 1987; 136:1163-67.

18. Klein JJ, Lefkowitz MS, Spector SL, Cherniack RM. Relationship between serum theophylline levels and pulmonary function before and after inhaled beta-agoinst in "stable" asthmatics. Am Rev Respir Dis 1983; 127:413-6.

19. Fairshter RD, and Busse WW. Theophylline - How much is enough? J Allergy Clin Immunology 1986; 77:646-8.

CHAPTER 8

CHANGES IN BRONCHIAL HYPERRESPONSIVENESS INDUCED BY 4 WEEKS OF TREATMENT WITH ANTI-ASTHMATIC DRUGS IN ALLERGIC ASTHMATIC PATIENTS; A COMPARISON BETWEEN BUDESONIDE AND TERBUTALINE

J. Kraan[1], G.H. Koëter[1], Th.W. v.d. Mark[2], H.J. Sluiter[1] and K. de Vries[3]

Departments of Pulmonology[1], Lung function[2] and Allergology[3], Clinic of Internal Medicine, University of Groningen, the Netherlands

Summary

We performed a double-blind crossover study to compare the effects of long-term treatment of inhaled budesonide and terbutaline on bronchial hyperresponsiveness in 17 allergic asthmatic patients. Both drugs were given for 4 weeks, with a placebo-treatment period before and after each active treatment period. To assess bronchial hyperresponsiveness, standardized inhalation provocation tests with histamine and propranolol were performed every 2 weeks. Before each inhalation provocation the drugs were withheld for at least 12 hours.

Before the budesonide treatment the FEV_1 value (% predicted) was 85.3 ±4.1% (mean ±SEM). After 2 and 4 weeks of treatment with this drug this value increased significantly to 89.4 ±4.1 and 96.2 ±3.8 respectively ($p<0.05$ and $p<0.005$). The histamine provocation concentrations causing a decrease in FEV_1 of 20% (PC_{20}) on the same days were 4.0, 7.2 and 9.5 mg.mL^{-1} respectively (both $p<0.001$). The PC_{20} values for propranolol, which were measured 1 hour after the histamine provocation, were 11.7, 13.3 and 14.0 mg.mL^{-1} (n.s.).

The FEV_1 values before and after 2 and 4 weeks of treatment with terbutaline were 86.2 ±4.0, 84.8 ±4.1 and 87.0 ±4.6% respectively. The histamine PC_{20} values on the same days were 4.7, 3.1 ($p<0.05$) and 3.8 mg.mL^{-1} respectively. the propranolol PC_{20} values, were 14.2, 8.7 and 10.1 mg.mL^{-1} ($p<0.001$ and $p<0.05$ respectively).

We conclude that budesonide improves bronchial hyperresponsiveness, possibly by a dampening of late allergic reactions, whereas treatment with terbutaline may lead to a temporary increase of bronchial hyperresponsiveness possibly due to beta-receptor desensitization.

J Allergy Clin Immunol 1985; 76:628-39

Introduction

Bronchial hyperresponsiveness, a common feature of asthma, can be assesed by inhalation provocation tests with agents such as histamine and propranolol[1]. When standardized methodes are used, these tests may provide good information about the severity of the disease[2]. By performing inhalation provocation tests with both bronchial obstructive agents, we investigated whether maintenance treatment with terbutatine and budesonide, administered by inhalation four times daily during four weeks, may change the degree in bronchial hyperresponsiveness. Both drugs are widely used anti-asthmatics with a different mode of action.

Terbutaline is a potent bronchodilator with usually minor dose dependent side effects such as tremor. Prolonged administration of this drug may result in beta-receptor desensitization[3,4]. It remains, however, to be established whether this phenomenon has any clinical relevance in regard to a possibly diminished bronchial response after administration of beta-agonists, or to an increase in bronchial hyperresponsiveness after withdrawal of these drugs[5]. In a previous study we observed that inhalation provocation tests with propranolol could demonstrate changes in bronchial hyperresponsiveness induced by treatment with terbutaline[6].

Budesonide is a recently developed corticosteroid with a strong topic effect on the bronchial mucosa, combined with a rapid inactivation in the systemic circulation[7]. Administration of corticoteroids causes an inhibition of late broncial responses to allergen challenge and dampening of inflammatory processes[8]. Both late bronchial responses on allergen challenges and inflammatory processes may cause an increase in hyperresponsiveness possibly caused by an increase in epithelial permeability[9]. Prolonged administration of budesonide may therfore cause a lower degree in bronchial responsiveness by restoring the increased permeability. In this study both drugs (terbutaline and budesonide) were administered during 4 weeks four times daily in a double-blind crossover fashion. Before and after each active treatment period, the patients went through a placebo treatment period. Before, during, and after each treatment period the degree of bronchial hyperresponsiveness was measured every 2 weeks by inhalation provocation tests with histamine and propranolol. The provocation tests were carried out after withdrawal of the active medication for at least 12 hours. In addition to the inhalation provocation tests, we also assessed the severity of the complaints and measured daily variation in peak flow rates. To test the possibility that effects of budesonide on bronchial hyperresponsiveness were caused by an effect on the allergic process, we also did repeated eosinophil counts of the peripheral blood.

Patients and methods

Patients

Fifteen male and 5 female patients with allergic asthma (mean age 25 years; range 18 to 38 year), gave their informed consent to participate in the study. The clinical characteristics of each patient are illustrated in table I. All patients were selected on a history of episodic wheezing, strongly positive skin tests (Diephuis laboratories, Groningen, the

Netherlands) for at least two common allergens, including house-dust mite (16 patients had late positive skin tests as well). All patients had mild symptoms, which could be controlled by a small dose of an inhaled bronchodilator or a prophylactic drug (disodium chromoglycate or inhaled corticosteroids).

Table 1. Clinical data of the patients.

Patient no.	Age (yr)	VC (%pred)	FEV_1 (%pred)	Histamine PC_{20}(mg.mL^{-1})	House Dust Mite Allergy: Skin Tests early	Skin Tests late	Spec. IgE IU.mL^{-1}	Maintenance drugs before trial
1	23	85	92	2.1	+	+	10-20	DSCG, iab
2	27	85	76	5.5	+	-	2-5	DSCG, becl., iab
3	31	95	79	2.3	+	+	10-20	DSCG
4	27	100	91	4.5	+	-	10-20	-
5	22	95	90	5.5	+	+	10-20	DSCG, terb.
6	20	90	100	12.8	+	+	20	DSCG, iab
7	22	97	75	6.9	+	+	10-20	DSCG, terb.
8	20	112	80	7.0	+	+	20	DSCG, thiaz.
9	18	108	85	1.0	+	+	10-20	DSCG, iab
10	32	120	77	22.5	+	+	20	DSCG, thiaz.
11	35	105	98	2.4	+	+	20	DSCG, thiaz.
12	20	95	90	11.2	+	+	20	DSCG, iab
13	23	98	80	2.1	+	+	10-20	-
14	27	85	78	3.6	+	+	20	DSCG, iab
15	22	104	100	9.3	+	-	20	DSCG
16	23	120	95	1.5	+	+	20	DSCG, becl.
17	20	86	80	4.7	+	-	20	-
18	24	92	95	4.2	+	+	20	DSCG, becl., iab
19	38	84	75	3.7	+	+	20	DSCG, thiaz.
20	27	85	76	5.6	+	+	10-20	DSCG, iab

Histamine and propranolol PC_{20}: see methods. The skin tests were recorded after 15 min and after 6 hours. VC= vital capacity; DSCG= disodium chromoglycate; iab= ipratropium bromide; becl.= beclomethason; terb.= terbutaline; thiaz.= thiazinamium.

The patients were further characterized by eosinophilia of the peripheral blood and positive IgE-RAST tests for house dust mite. The initial forced expiratory volume in one second (FEV_1) was not below 75% of the predicted value; bronchial obstruction could be completely reversed by 12.5 mg thiazinamium intramuscularly.

All patients had an increased bronchial responsiveness to inhaled histamine, defined by a histamine PC_{20} below 32 mg.mL^{-1} (see methods, inhalation provocation tests).

Study design

A two-treatment double-blind crossover study during 126 days was performed. Patients were randomly allocated to treatment group A or B (see fig. 1).

Budesonide and terbutaline were both administered by a metered dose inhaler (m.d.i.) four times a day in a dose of 100 and 500 microgram respectively. The terbutaline m.d.i. produced 250 µg per puff and the budesonide m.d.i. produced 50 µg per puff. Placebo inhalations were also taken four times a day.

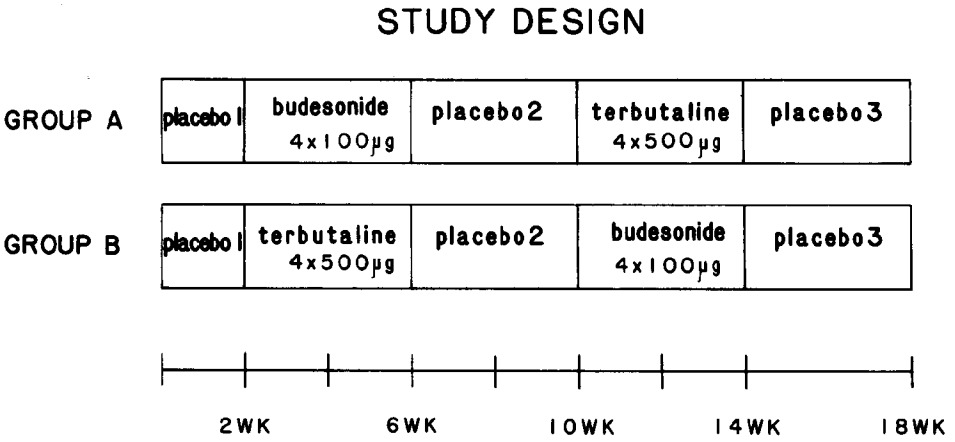

Figure 1. A two-treatment, double blind, crossover study. Patients were randomly allocated.

Measurements were made at the start and subsequently every 2 weeks until the last day of the trial. The measurements were carried out between 12 and 16 hours after the last drug inhalation and were always performed at the same time of the day.

Before entering the study the patients stopped their maintenance drugs for at least 3 weeks, using only ipratropium bromide inhalations to control their symptoms. Therefore, when patients came into the first active treatment period, they had not been receiving their maintenance treatment for at least 5 weeks. A possible effect of this previous maintenance treatment on the test results is therefore unlikely. Most patients were studied during the months December to April, thus preferentially outside the allergic season, although most patients had no specific seasonal complaints.

At each clinical visit lung function was tested, and histamine and propranolol inhalation provocation tests were performed. At each visit blood was collected for eosinophil count. The drug cannisters were weighed at each visit to assess patients treatment compliance.

The protocol was approved by the hospital ethical commitee.

Pulmonary function and inhalation provocation tests

Slow inspiratory vital capacity (VC) and FEV_1 were measured with a water sealed spirometer. The data were expressed in percentages of the predicted values[10]. At home, peak flow was recorded twice a day during the whole study before drug inhalation. These data were also expressed in percentages of the predicted values.

The histamine and propranolol inhalation provocation concentration was measured by inhaling aerosols with increasing concentrations of the agents. The solutions were nebulished with a "Wiesbadener doppelspray" with an airflow of 8 liters.min^{-1}. The output of the nebuliser is within 0.120±0.013 mL.min^{-1}. The aerosols were inhaled at tidal breathing in a semi-closed system. The histamine concentration ranged from 1 mg.mL^{-1} to 32 mg.mL^{-1}, concentration steps being 1, 2, 4, 8, 16, 32 mg.mL^{-1} respectively. Each step was inhaled for 30 sec, at 5 minute intervals, until PC_{20} compared with a control inhalation or

until the maximal concentration was inhaled[11]. Propranolol concentrations were 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 mg.mL^{-1}. Each concentration step of propranolol was inhaled for 2 min. FEV_1 was measured immediately after the inhalation and after 2 minutes. The provocation concentration of histamine and propranolol required to produce a fall in FEV_1 of 20% was calculated from the dose-response curve (PC_{20} values).

Symptom scores

At every visit at the outpatient clinic patients were questioned about asthmatic attacks, wheezing, shortness of breath on exercise and coughing. These four complaints were scored quantitatively by the patient along a scale from 1 to 4 (1 = severe complaints, 4 = no complaints). The scores were added to form a general score (minimal=4, maximal=16).

Statistical analysis

Logarithmic transformation of the histamine PC_{20} was used for statistical calculations. In the given dose range for propranolol (with linearly increasing dose steps) a linear function is a sufficient approximation for the dose-response curve. Therefore the propranolol PC_{20} was not logarithmically transformed.

Values of PC_{20}'s, peak flow, symptom scores, etc., during active treatment were compared with values during placebo just before the active treatment periods (e.g. comparison of day 15 with day 29 and 43; and day 71 with day 85 and 99) by use of Student's t-test for paired values.

By contrast, to compare differences between the effects of the two active treatments, budesonide and terbutaline, multivariate analysis of variance (MANOVA) was performed on the data. This includes testing on sequence effects and residual effects of the first treatment on the second given treatment[12,13]. All the data are expressed in mean ± SEM

Dropouts

Of the original 20 patients who started the trial, three had to stop the protocol because of increasing asthmatic symptoms during placebo-treatment period 1. For this reason patient numbers in treatment group A and B were not equal. Of the 17 patients who were finally included in the study 10 performed treatment sequence A (budesonide-terbutaline) and 7 performed treatment sequence B (terbutaline-budesonide). Of these 17 patients, 15 completed the study as far as the second active-treatment period. Two patients dropped out during the second active-treatment period. Of these two patients the results of initial lung function and bronchial provocation tests after 2 weeks in the second active-treatment period are available, whereas the results after 4 weeks of treatment are not available. Of these two patients one had a viral upper airway infection with increased asthmatic symptoms and the other patient used a wrong drug cannister, caused by a distribution failure.

Five patients did not complete placebo period 3. Two of these patients dropped out because of a viral infection, with increased asthmatic symptoms when they ware halfway this period. The other 3 patients could not complete the study because of holidays, study-courses, etc. No patients were omitted caused by lack of treatment compliance.

Results

Placebo periods and residual effects

The mean FEV_1 values (for all patients) at the end of placebo periods 1 and 2 were 84.7 ± 4.2% and 86.9 ± 4.0% predicted, respectively. The histamine PC_{20} values measured on the same days were 4.8 and 3.9 $mg.mL^{-1}$ (expressed in logarithmic values 0.68 ± 0.10 and 0.60 ± 0.10, respectively). The data were not significantly different from each other.

With MANOVA the possibility that residual effects of one treatment on the other existed, could be rejected ($p>0.2$). Therefore, it is justified to pool the data of the active-treatment periods budesonide vs terbutaline of both patient groups without taking the treatment sequences into account.

Effects of budesonide compared to placebo

Initial FEV1 values (fig. 2a) The pretreatment FEV_1 was 85.3 ± 4.1%. Treatment with budesonide during 2 and 4 weeks caused a significant increase to 89.4 ± 4.1% and 96.2 ± 3.8% respectively ($p<0.05$ and $p<0.005$).

The values returned to pretreatment levels during the washout period.

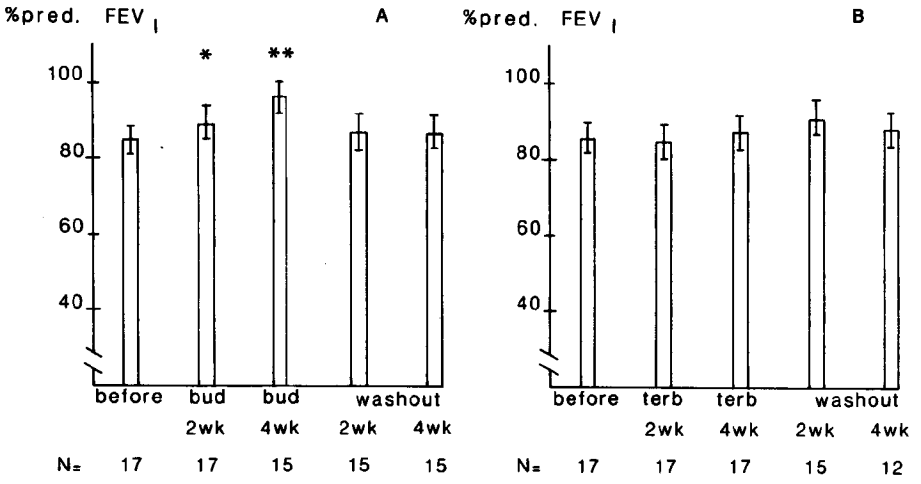

Figure 2. A, The effect of treatment with budesonide and B, terbutaline on initial FEV_1. The FEV_1 (% predicted) measured during and after treatment is statistically compared with the FEV_1 before treatment (*, $p<0.05$; **, $p<0.005$). Bars indicate ± SEM; bud, budesonide; terb, terbutaline; N, number of observations.

Histamine PC_{20} (fig. 3a) Before the budesonide treatment was started, the histamine PC_{20} value was 4.0 $mg.mL^{-1}$, after 2 and 4 weeks of treatment this value increased to 7.2 and 9.5 $mg.mL^{-1}$ respectively (expressed in log values: 0.60 ± 0.09; 0.86 ± 0.10; 0.98 ± 0.10). Both changes were significantly different from the pretreatment value ($p<0.001$).

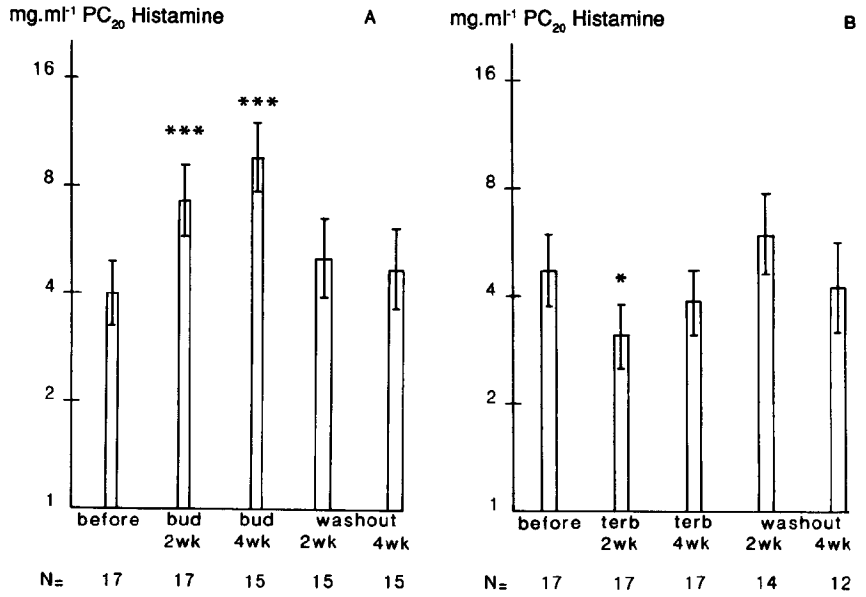

Figure 3. A, The effect of treatment with budesonide and B, terbutaline on histamine PC_{20} (milligrams per milliliter). The histamine PC_{20} measured during and after treatment is statistically compared with the PC_{20} before treatment (*, $p<0.05$; ***, $p<0.001$). Bars indicate ± SEM; bud, budesonide; terb, terbutaline; N, number of observations.

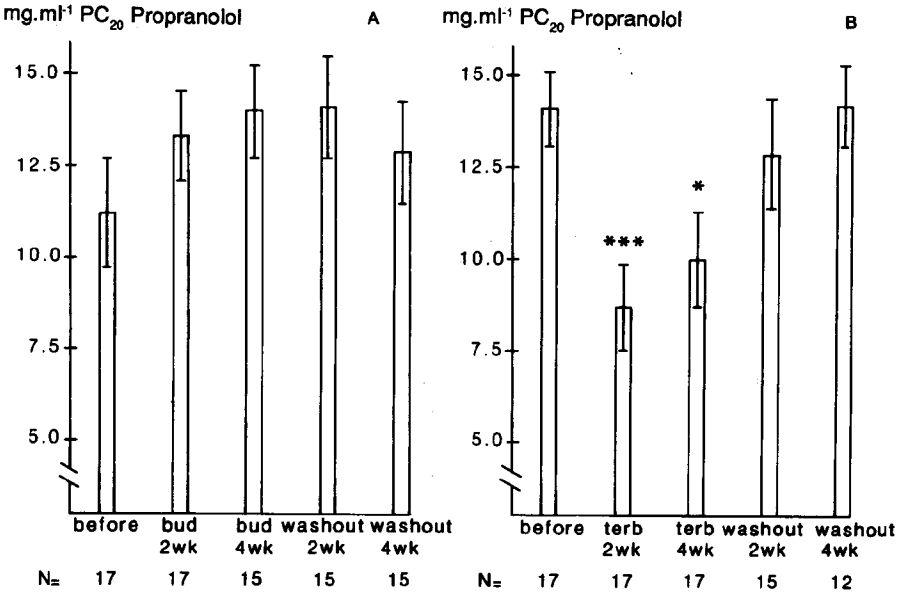

Figure 4. A, The effect of treatment with budesonide and B, terbutaline on propranolol PC_{20} (milligrams per milliliter). The propranolol PC_{20} measured during and after treatment is statistically compared with the PC_{20} before treatment (*, $p<0.05$; ***, $p<0.001$). Bars indicate ± SEM; bud, budesonide; terb, terbutaline; N, number of observations.

Propranolol PC_{20} (fig. 4a) The pretreatment propranolol PC_{20} value was 11.7 ± 1.5 mg. mL^{-1}. After treatment (2 and 4 weeks) these values were 13.3 ± 1.3 mg.mL^{-1} and 14.0 ± 1.3 mg.mL^{-1}, respectively (n.s.).

Peak flow rates (fig. 5a) During budesonide treatment the morning and evening peak flow rates were increased after 2 and 4 weeks of treatment, compared with the placebo period, whereas the diurnal variation decreased. The morning values were 81.9 ± 4.0%, 88.7 ± 4.0% and 90.5 ± 4.0% predicted respectively (both $p<0.001$). The evening peak flow values were 89.4 ± 4.0%, 94.7 ± 3% and 95.0 ± 4.0% (both $p<0.005$). The diurnal variation values were 7.5 ± 1.6%, 6.1 ± 1.4% (n.s.) and 4.5 ± 1.2% respectively ($p<0.05$).

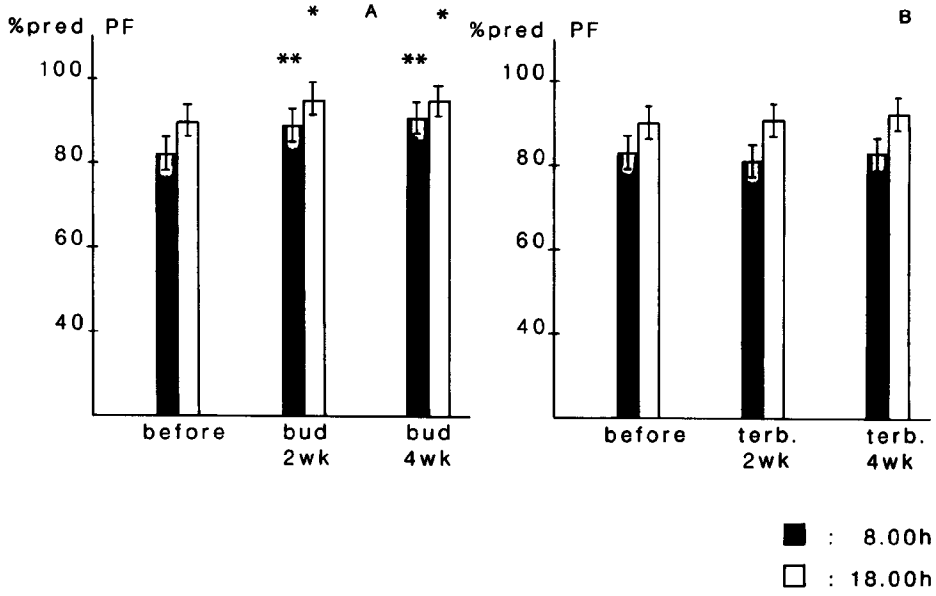

Figure 5. A, The effect of treatment with budesonide and B, terbutaline on peak flow rate. The peak flow measurements (% predicted) were carried out before drug inhalation (8.00 and 18.00 hours). The values during active treatment are compared with the pretreatment value (*,$p<0.005$, **,$p<0.001$). Bars indicate ± SEM; bud, budesonide; terb, terbutaline.

Symptom scores Mean values for symptom scores changed from 12.5 ± 0.6 before treatment to 15.3 ± 0.3 (2 weeks of treatment) and to 15.5 ± 0.3 (4 weeks of treatment, $p<0.01$).

Eosinophil counts The eosinophil count decreased from 305 ± 43.mm^{-3} before treatment to 242 ± 46.mm^{-3} after 4 weeks of treatment (n.s.).

Values after 2 weeks compared to values after 4 weeks of treatment
The values of FEV_1, PC_{20}'s, peak flow rates and symptom scores after 2 weeks of treatment were not significantly different from the values after 4 weeks of treatment.

Effects of terbutaline compared to placebo

Initial lung function (fig. 2b) Initial FEV_1 remained stable during terbutaline therapy. The values before and after 2 and 4 weeks of treatment were 86.2 ± 4.0%, 84.8 ± 4.1% and 87.0 ± 4.6% respectively.

Histamine PC_{20} (fig. 3b) During 2 weeks of terbutaline treatment the mean value of histamine PC_{20} decreased from 4.7 to 3.1 $mg.mL^{-1}$ ($p<0.05$). After 4 weeks of treatment this provocation concentration changed to 3.8 $mg.mL^{-1}$ (n.s.). Expressed in logarithms these values are 0.67 ± 0.11, 0.49 ± 0.09 and 0.58 ± 0.09, respectively. During the washout period, the PC_{20} increased again.

Propranolol PC_{20} (fig. 4b) The pretreatment value of the propranolol PC_{20} was 14.2 ± 1.1 $mg.mL^{-1}$. After 2 and 4 weeks of treatment, it changed to 8.7 ± 1.2% and 10.1 ± 1.3 $mg.mL^{-1}$ respectively. These changes were statistically significant ($p<0.001$ and $p<0.05$). During the washout period, the values returned to the pretreatment levels.

Peak flow rates (fig. 5b) During terbutaline treatment, morning and evening peak flow rates did not change significantly. However, morning-evening variations rose significantly from 6.8 ± 1.0% to 9.5 ± 1.6% after 2 weeks of treatment ($p<0.05$) and to 10.0 ± 1.3% after 4 weeks of treatment ($p<0.005$).

Symptom scores The symptom scores remained stable during terbutaline treatment compared to pretreatment values. The values were 12.6 ± 0.6, 12.5 ± 0.5 and 13.1 ± 0.5 respectively.

Eosinophil counts The eosinophil count changed from 389 ± 72.mm^{-3} before treatment to 363 ±63.mm^{-3} after 4 weeks of treatment (n.s.).

Values after 2 weeks compared to values after 4 weeks of treatment For all measured parameters we observed no significant differences between 2 and 4 weeks of treatment.

Differences between effects of budesonide and terbutaline treatment

The effects of 2 weeks and 4 weeks of treatment with budesonide and terbutaline on initial FEV_1, histamine PC_{20}, propranolol PC_{20}, peak flow rates, symptom scores and eosinophil counts are compared in table 2. The MANOVA demonstrated that the treatment effect was the only significant contribution to the observed differences. There were no significant sequence effects or residual effects of one of both treatment on the other.

Histamine PC_{20}, propranolol PC_{20}, peak flow values and symptom scores were significantly higher after 2 weeks of budesonide treatment than after terbutaline treatment. Diurnal peak flow variation and eosinophil counts were significantly lower on budesonide treatment.

After 4 weeks of treatment the initial FEV_1, histamine PC_{20}, morning peak flow rates and symptom scores were significantly higher on budesonide treatment than on terbutaline treatment. Also the diurnal peak flow variation and eosinophil counts were significantly lower on budesonide treatment.

Table 2. Comparison of effects of prolonged treatment with inhaled budesonide and terbutaline.

	After 2 weeks (n=17)		After 4 weeks (n=15)	
	Budesonide	Terbutaline	Budesonide	Terbutaline
Initial FEV_1 (% pred.)	89.4 ± 4.1	84.4 ± 4.1	96.2 ± 3.8	89.9 ± 4.6*
Histamine PC_{20} ($mg.mL^{-1}$)	7.2	3.1	9.5	4.1
Logarithmic value	0.86± 0.10	0.49± 0.09‡	0.98 ± 0.10	0.61 ± 0.10**
Propranolol PC_{20} ($mg.mL^{-1}$)	13.3 ± 1.3	9.1 ± 1.2**	14.0 ± 1.3	11.0 ± 1.3
Peak flow (% pred.)				
8 hours	88.7 ± 4.0	81.1 ± 3.4‡	90.5 ± 4.1	83.4 ± 4.2‡
18 hours	94.7 ± 3.0	90.5 ± 3.0*	95.5 ± 4.0	93.3 ± 3.4
diurnal variation	6.1 ± 1.4	9.5 ± 1.5*	4.5 ± 1.2	10.0 ± 1.3**
Symptom score	15.3 ± 0.3	12.4 ± 0.5**	15.2 ± 0.2	13.2 ± 0.7**
Eosinophil count ($.mm^{-3}$)	253 ± 36	366 ± 61*	242 ± 46	363 ± 63**

N=number of patients. budesonide was administered in a dosage of 100 µg 4 times daily; terbutaline was administered in a dosage of 500 µg 4 times daily. for measurements and calculations of histamine PC_{20} and propranolol PC_{20} and calculation of symptom score, see Methods section. Statistical analysis was done by MANOVA.

Data are illustrated as mean ± SEM. because the results after 4 weeks of terbutaline are based on data of 15 patients, the figures are not exacly the same as in the text where the results compared with placebo are based on data of 17 patients.

* $p<0.05$; ** $p<0.01$; ‡ $p<0.001$.

Discussion

The results of the present study demonstrate that maintenance treatment with inhaled budesonide may lead to an improvement in bronchial hyperresponsiveness and in pulmonary function and to a better symptom score compared with values obtained during placebo-tratment periods. Treatment with inhaled terbutaline induces a temporary increase in bronchial hyperresponsiveness that almost disappears in the course of further maintenance treatment. When terbutaline was withheld for at least 12 hours, no significant changes in pulmonary function were observed compared with the placebo-treatment period (treatment with terbutaline did not lead to changes in pulmonary function). The symptom scores on terbutaline treatment were not significantly better than during the placebo-treatment period.

There are only a few reports on the investigation of long-term effects of inhaled corticosteroids on the degree of bronchial hyperresponsiveness. Easton[14] found no significant changes of methacholine airway responsiveness after 4 months treatment with beclomethasone dipropionate (BDP) in asthmatic patients, although patients tended to improve slightly. Juniper et al.[15] followed bronchial hyperresponsiveness in patients with asthma up to 30 months. The main conclusion of their study was that bronchial hyperresponsiveness remains stable over long periods when there are no exacerbating factors. These authors also found that in a sub-group of their patients, bronchial hyperresponsiveness to

histamine tended to improve after prolonged treatment with inhaled corticosteroids. The latter study was however carried out without a control group.

In a recent study, Henrikson and Dahl[16] found a benificial effect of inhaled budesonide on exercise-induced asthma in children. The fall in FEV_1 after exercise seems to be strongly correlated to histamine PC_{20} values, which suggests that exercise-induced asthma is a phenomenon of increased bronchial responsiveness[17]. Therefore, our results appear to be in agreement with those of Henrikson and Dahl.

In the present study, an improvement in initial lung function as well as an improvement in bronchial hyperresponsiveness after treatment with budesonide was observed. It can not be excluded that the decrease in bronchial hyperresponsiveness is partially caused by an improvement in pulmonary function because it is well known that both parameters are weakly correlated[18]. It is unlikely, however, that the results on the change in bronchial hyperresponsiveness in our patient group are markedly affected by the changes in initial FEV_1 values, because the FEV_1 was not less than 75% of the predicted value[19]. Moreover, we found no significant correlation between the improvement in FEV_1 and change in histamine PC_{20} during the budesonide-treatment period ($r = 0.15$). It might therefore be concluded that inhaled corticosteroid drugs improve bronchial hyperresponsiveness in patients with allergic asthma.

The underlying mechanism is unclear. The observed phenomena can possibly be explained by an inhibition of late allergic responses in our patient group. Many patients with extrinsic asthma have late bronchial obstrutive responses caused by allergen exposure[20,21], which can induce an increase in bronchial hyperresponsiveness[22].

Treatment with corticosteroid drugs can prevent the occurrence of late bronchial obstructive reactions to allergens[23]. It is therefore quite conceivable that treatment with corticosteroids of patients with extrinsic asthma, in whom these late asthmatic reactions occur leads to improvement of their bronchial responsiveness. Maintenance treatment with corticosteroids may inhibit processes like the formation of mediators with inflammatory properties, leading to a repair of the damaged airway epithelium[8,9].

The slight trend to decrease of the eosinophil count in our patients when they were treated with budesonide indeed points to this inhibition of the allergic process[24]. Whether further improvement of bronchial hyperresponsiveness in extrinsic asthmatic patients can be effectuated by a larger dose of inhaled corticosteroid drugs and a more prolonged use than in our study is a question for further research.

Our study demonstrated a slight increase in bronchial hyperresponsiveness to histamine and propranolol inhalations after 2 weeks of maintenance treatment with inhaled terbutaline, but the increased responsiveness to histamine had almost disappeared when the therapy was continued to 4 weeks.

Whether beta-adrenergic drugs may cause a decrease in beta-receptor responsiveness, leading to a decrease in bronchial response to that drug, is still under discussion[5]. Moreover, an impairment of beta- receptor function may lead to an increase in bronchial responsiveness, which may cause an increase in histamine PC_{20} values.

Only a few studies have been published on this subject. Peel and Gibson[25] found no change in histamine challenges after 4 weeks of regular treatment with salbutamol 4 times per day by aerosol. These results are in agreement with the observation of Tashkin et al.[3] who measured histamine bronchial responsiveness 3 to 5 weeks after oral maintenance

treatment with terbutaline. We found a slight but significant increase in hyperresponsiveness to inhaled histamine after two weeks of treatment with terbutaline, but this hyperresponsiveness to histamine almost returned to initial values when terbutaline treatment was continued up to 4 weeks. The observed change in bronchial hyperresponsiveness could not be explained by a change in FEV_1.

Inhalation of propranolol leads to a bronchial obstruction in subjects with asthma[26] and bronchial provocation with this agent appears appropriate to test decreased beta-receptor function in the bronchial smooth muscle. In a previous study we noted an increased sensitivity to inhaled propranolol, together with a subsensitization of beta-receptors on lymphocytes after 2 weeks of treatment with oral terbutaline[6].

In the present study an increase in bronchial responsiveness to inhaled propranolol was demonstrated both after 2 and 4 weeks of treatment. This appears to implicate that a possible decreased beta-receptor function leading to an increased bronchial hyperresponsiveness to inhaled agents is more easily demonstrated with propranolol than with histamine. In a recent study of Vathenen et al.[27] our finding of an increased BHR to histamine after 2 weeks of maintenance treatment with an inhaled beta-agonist has been confirmed. In addition, these authors demonstrated a decrease in the protective effect of terbutaline against histamine-induced bronchoconstriction (when the drug was administrated immediately before histamine provocation).

When both active treatment periods in our study are compared, a pronounced difference between the effect of 4 weeks of therapy with budesonide and terbutaline especially on bronchial hyperresponsiveness and to a lesser extend on initial FEV_1 on the bronchial provocation days, can be observed. This difference is also seen in the results of the daily home peak flow measurements and in the symptom scores. Treatment with terbutaline leads to a slight increase of diurnal variations in peak flow rates. There is a slight increase of evening peak flow rates during terbutaline treatment compared to the measurements during the placebo-treatment period. This could be expected because the direct bronchodilatory effect after inhalation lasts 4 to 6 hours and the evening measurements were done 4 to 6 hours after the last drug inhalation. In contrast to treatment with terbutaline, treatment with budesonide leads to increased morning values of peak flow rates and decreased diurnal variations. This change is also reflected in the improvement of symptom scores. Although terbutaline is a strong bronchodilatory agent, the patients in our study experience more symptoms like wheezing, morning dyspnea and dyspnea after exercise on prolonged single therapy with terbutaline than with budesonide.

We conclude that prolonged use of inhaled budesonide is superior to inhaled terbutaline in patients with extrinsic asthma. Treatment with budesonide leads to an improvement of bronchial hyperresponsiveness, improvement in FEV_1, morning and evening peak flow rates, decrease of diurnal variation of peak flow values and improvement of symptom scores. Terbutaline treatment leads to a, probably temporarily, increase of bronchial hyperresponsiveness, which, in the light of the strong bronchodilatory quality of the drugs seems to be of little clinical relevance. It appears to be justified to prescribe inhaled corticosteroids next to bronchodilators in patients with mild (allergic) asthma, because corticosteroids may change bronchial hyperresponsiveness.

References

1. Boushey HA, Holtmann MJ, Sheller JR, Nadel JA: Bronchial hyperreactivity. State of the Art. Am Rev Respir Dis 1980; 121:389-413.
2. Juniper EF, Frith PA, Hargreave FE: Airway responsiveness to histamine and methacholine; relationship to minimum treatment to control symptoms of asthma. Thorax 1981; 36:575-9.
3. Tashkin DP, Conolly ME, Deutsch RI, Kit Hui K, Littner M, Scarpace PH, Abrass I: Subsensitization of beta-adrenoceptors in airways and lymphocytes of healthy and asthmatic subjects. Am Rev Respir Dis 1982; 125:185-93.
4. Van den Berg W, Leferink JG, Fokkens JK, Kreukniet J, Maes RAA, Bruynzeel PLB: Clinical implications of drug-induced desensitization of the beta-receptor after continuous oral use of terbutaline. J Allergy Clin Immunol 1982; 69:410-7.
5. Jenne JW, Wither beta-adrenergic tachyphyaxis. J Allergy Clin Immunol 1982; 70:413-6.
6. Koëter GH, Meurs H, Kauffman HF, De Monchy JGR, Sluiter HJ, De Vries K: Changes in the beta-adrenergic system in bronchial asthma induced by terbutaline. Agents and Actions 1983; 13 suppl:259-64.
7. Johansson SA, Andersson KE, Brattsand R, Gruvstad E, Hedner P: Topical and systemic glucocorticoid potencies of budesonide, beclomethason diproprionate and prednisolone in man. Eur J Respir Dis 1982; 63, suppl 122:74-82.
8. Clark TJH, McAllister WAC: Corticosteroids. In: Asthma, Edited by Clark TJH and Godfrey S, 2nd ed., London, Chapman and Hall, 1983:372-92.
9. Hogg JC: Bronchial mucosal permeability and its relationship to airway hyperreactivity. J Allergy Clin Immunol 1981; 67:421-5.
10. Tammeling GJ: Standard values for lung volume and ventilatory capacity of sanatorium patients. Selected papers, Royal Netherlands Tuberculosis Association, 1961; 1:65.
11. De Vries K, Goei JT, Booy-Noord H, Orie NGM: Changes during 24 hours in the lung function and histamine hyperreactivity of the bronchial tree in asthmatic and bronchitic patients. Int Arch All 1962; 20:93-101.
12. Grizzle JE: The two-period change-over design and its use in clinical trials. Biometrics 1965; 21:467-80.
13. Nie NH, Hull Ch, Jenkins JG, Steinbrecher K, Bent DH: SPSS Handbook, Releases 7-9, 1981.
14. Easton JG: Effect of an inhaled corticosteroid on methacholine airway reactivity. J Allergy Clin Immunol 1981; 67:388-90.
15. Juniper EF, Frith PA, Hargreave FE: Long term stability of bronchial responsiveness to histamine. Thorax 1983; 37:288-91.
16. Henriksen JM, Dahl R: Effects of inhaled budesonide alone and in combination with low-dose terbutaline in children with excercise- induced asthma. Am Rev Respir Dis 1983; 128:993-7.
17. Neyens HJ, Wesselius T, Kerrebijn KF: Exercise induced bronchoconstriction as an expression of bronchial hyperreactivity: a study of its mechanism in children. Thorax 1981; 36: 517–22.
18. Benson MK: Bronchial Hyperreactivity. Brit J Dis Chest 1975; 69:227-39.
19. Ryan G, Latimer KM, Dolovich J, Hargreave FE: Bronchial responsiveness to histamine: relationship to diurnal variation of peak flow rate, improvement after bronchodilator and airway calibre. Thorax 1982; 37:423-9.

20. Kaliner M: Editorial: Hypotheses on the contribution of late-phase allergic responses to the understanding and treatment of allergic diseases. J Allergy Clin Immunol 1984; 73: 311-6.

21. Cockcroft DW: Hypothesis: Mechanism of perennial allergic asthma. Lancet 1983; II: 253–6.

22. Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE: Allergen induced increase in non-allergic bronchial reactivity. Clin Allergy 1977; 7:503-13.

23. Booy-Noord H, Orie NGM, De Vries K: Immediate and late bronchial obstructive reactions to inhalation of house dust and protective effects of disodium cromoglycate and prednisolone. J Allergy Clin Immunol 1971; 48:344-54.

24. De Monchy JGR, Kauffman H, Venge P, Koëter GH, Jansen HM, Sluiter HJ, De Vries K: Broncho-alveolar eosinophilia during allergen-induced late asthmatic reactions. Am Rev Respir Dis 1985; 131:373-6.

25. Peel ET, Gibson GJ: Effects of long-term inhaled salbutamol therapy on the provocation of asthma by histamine. Am Rev Respir Dis 1980; 121:973-8.

26. MacDonald AG, Ingram CG, McNeill RS: The effect of propranolol on airway resistance. Br J Anaesth 1976; 39:919-23.

27. Vathenen AS, Knox AJ, Higgins BG, Britton JR, Tattersfield AE. Rebound increase in bronchial responsiveness after treatment with inhaled terbutaline. Lancet 1988; 1:554-8.

CHAPTER 9

DOSAGE AND TIME EFFECTS OF INHALED BUDESONIDE ON BRONCHIAL HYPERRESPONSIVENESS

J. Kraan, G.H. Koëter, Th.W. v.d. Mark, M. Boorsma, J. Kukler, H.J. Sluiter, K. de Vries

From the Departments of Pulmonology, Lung Function, and Allergology, Clinic of Internal Medicine, University of Groningen, and Astra Pharmaceutica BV, Rijswijk, the Netherlands

Summary

In a double-blind study of 2 parallel groups of 15 allergic asthmatic patients each, we investigated whether treatment with inhaled budesonide has a dose- and time-dependent effect on the degree of bronchial hyperresponsiveness. The patients were randomly allocated to treatment with either 200 or 800 μg budesonide per day for a period of 8 wk. The active treatment period was preceded by a selection period of 3 wk, and a single blind placebo period of 2 wk. During these initial 5 wk the maintenance treatment of the patients, including cromolyn sodium and inhaled corticosteroids, was withheld. Spirometry and inhalation provocation tests with methacholine were carried out and the symptom score was recorded every 2 wk. The methacholine provocation concentrations (geometric mean) causing a decrease in FEV_1 of 20% (PC_{20}) in the 200 μg/day and 800 μg/day treatment groups just before the active treatment period were 0.90 and 0.91 mg.mL^{-1} respectively. These values increased significantly to 1.21 and 1.84 mg.mL^{-1} after 2 wk of treatment ($p<0.05$ and $p<0.001$ respectively) and to 1.55 and 2.74 mg.mL^{-1} after 8 wk of treatment, ($p<0.01$ and $p<0.001$). During the whole study period budesonide in a dosage of 800 μg/day induced a significantly larger change in PC_{20} than in a dosage of 200 μg/day. The FEV_1 before treatment was 91±3% (S.E.M.) and 84±2% of the predicted values in the two groups. The FEV_1 values after two wk of treatment were 96±3% and 93±2% respectively ($p<0.05$ and $p<0.001$). There was no further improvement in FEV_1 during the following 6 wk of treatment, in contrast to the PC_{20} values. We conclude that inhaled budesonide can diminish bronchial hyperresponsiveness in allergic asthmatic patients significantly and that this change is dose-dependant. Furthermore, the results indicate that the improvement in bronchial hyperresponsiveness is positively influenced by the duration of treatment.

Am Rev Respir Dis 1988; 137:44-8

Introduction

Bronchial hyperresponsiveness is a common feature of asthma[1]. It can be assessed by inhalation provocation tests with methacholine. When a standardized method is used, these tests provide information about the severity of the disease[2]. In recent studies a reduction in bronchial hyperresponsiveness has been demonstrated in asthmatic patients after prolonged treatment with inhaled corticosteroids[3,4]. This reduction in bronchial hyperresponsiveness is probably due to the effect of corticosteroids on the (allergic) inflammatory process[5].

In this study we investigated the possibility that maintenance treatment with an inhaled corticosteroid, budesonide, has a dose- and time-dependent effect on the degree of bronchial hyperresponsiveness. Budesonide is a recently developed corticosteroid for inhalation treatment with a strong local effect combined with a rapid inactivation in the systemic circulation[6]. The study was carried out in a group of allergic asthmatic patients, characterized by a mild bronchial obstruction (the FEV_1 before the study was > 70% of the predicted value). Two parallel groups were treated with either 200 or 800 μg of inhaled budesonide per day. These dosages can be considered as half and twice the recommended dose, respectively.

The degree of bronchial hyperresponsiveness was assessed by inhalation provocation tests with methacholine. Every 2 wk the patients came to the clinic for measurement of lung function, a methacholine provocation test and an assessment of symptoms. The study was carried out for 8 wk because we observed in a previous study that budesonide in a dose of 400 μg/day did not seem to reach its maximal effect after 4 wk of treatment[4]. Before and after the active treatment period the number of eosinophils in the peripheral blood and the serum cortisol level were measured.

In addition we investigated whether it was possible to predict the change in bronchial hyperresponsiveness induced by budesonide from pretreatment patient characteristics, such as initial methacholine PC_{20}, serum IgE against house dust mite, and number of blood eosinophils.

Patients and methods

Patients

Thirty (22 male and 8 female) patients with allergic asthma (mean age 27 yr; range 18 to 37 yr) gave their informed consent to participate in the study. The clinical characteristics of the patients are shown in Table 1.

All patients had a history of episodic wheezing, strongly positive skin tests (allergens: Diephuis laboratories, Groningen, the Netherlands) for at least 2 common allergens, including house dust mite. In addition, specific IgE levels against house dust mite were determined. All patients had mild symptoms, which were controlled by low dosages of inhaled bronchodilators or prophylactic drugs (cromolyn sodium or inhaled corticosteroids). None of the patients used oral corticosteroids regularly.

The initial FEV_1, measured during the selection period, was not below 70% of the predicted value[7]. All patients had an increased bronchial hyperresponsiveness to methacholine, defined by a methacholine PC_{20} below 8 $mg.mL^{-1}$ (see methods, Inhalation Provocation Tests).

Table 1. Clinical data of the patients.

Patient no	Age	FEV_1 * (%pred)	Methacholine PC_{20} ($mg.mL^{-1}$)	Serum IgE level against house dust mite ($iU.mL^{-1}$)	Maintenance drugs prior to trial
1	37	106	0.7	27.9	DSCG,BDP
2	21	98	1.3	36.0	BDP,iab
3	24	90	1.4	33.0	Terb
4	24	85	0.5	27.0	Iab
5	35	97	3.5	31.0	DSCG,terb
6	28	95	1.3	30.8	bud,iab
7	22	113	5.9	32.1	-
8	26	89	1.3	39.5	DSCG,terb,iab
9	20	103	0.9	38.6	DSCG,iab
10	34	89	0.6	0.28	DSCG,terb
11	27	103	1.4	30.8	DSCG
12	25	81	0.4	23.8	Cs,iab
13	24	115	1.9	27.2	-
14	37	78	0.4	7.3	DSCG,iab
15	28	89	0.9	21.8	-
16	22	91	2.4	8.1	-
17	31	93	2.4	0.10	DSCG,iab,bud
18	25	76	1.8	35.0	-
19	24	104	0.4	32.7	DSCG,bud,iab
20	26	101	3.0	26.6	DSCG,iab
21	24	101	0.4	32.7	Bud,terb
22	32	89	1.5	21.0	DSCG,BDP,iab
23	21	86	4.0	34.5	-
24	32	80	7.3	3.7	DSCG,iab
25	26	96	0.9	34.2	DSCG,iab
26	36	108	0.7	27.2	DSCG
27	20	89	4.6	21.5	DSCG,BDP,iab
28	18	78	0.7	37.6	-
29	28	104	2.3	23.9	DSCG,iab
30	23	85	0.7	27.2	Terb

Definition of abbreviations: DSCG= disodium chromoglycate Iab=ipratropium bromide; BDP=beclomethasone; Terb=terbutaline; Bud= budesonide.

* The FEV_1 and methacholine PC_{20} shown are the values that were recorded during the selection phase (before withdrawing the maintenance drugs). The serum IgE (rast) values are given as $iU.mL^{-1}$ (0.35 to 0.7 $iU.mL^{-1}$, slightly increased; 0.7 to 3.5 $iU.mL^{-1}$, moderately increased; 3.5 to 17.5 $iU.mL^{-1}$, strongly increased).

Study Design

In a double blind design, the asthmatic patients were randomly allocated to 1 of the 2 parallel groups. After a (single- blind)placebo treatment period of 2 wk 15 patients were treated with budesonide 200 µg/day (2 puffs containing 50 µg, twice daily) and 15 patients were treated with budesonide 800 µg/day (2 puffs containing 200 µg, twice daily). The study was carried out between January and June 1985.

Before entering the study, the patients discontinued their usual maintenance treatment for at least 3 wk (selection period). Therefore, when the patients started the active treatment period of the study, they had discontinued their previous maintenance treatment (including disodium chromoglycate or inhaled corticosteroids) for at least 5 wk. The patients were allowed to use inhaled ipratropiumbromide on an if necessary basis to control their possible symptoms during the whole study. All patients used this drug infrequently (less than once a day) and there was no change in the mean dose.

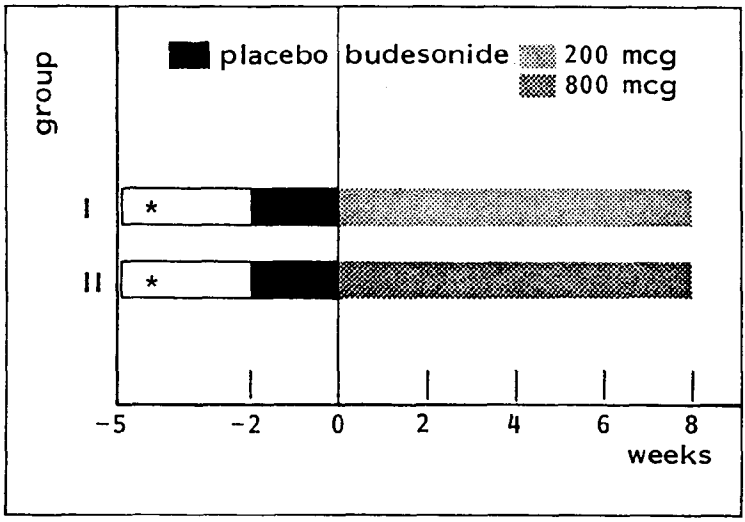

Figure 1. Study design. *: maintenance treatment was withheld.

The patients visited the clinic once during the selection period and thereafter every 2 wk during the study. During each clinical visit, spirometry was measured and a methacholine inhalation provocation test was performed. Before the start and at the end of the active treatment period blood was collected for eosinophil cell count. The drug cannisters were weighed at each visit to assess the treatment compliance of the patients.

The protocol was approved by the hospital medical ethical committee, and written consent from each patient was obtained.

Pulmonary function and methacholine inhalation provocation tests

Slow inspiratory vital capacity (VC) and forced expiratory volume in one second (FEV_1) were measured with a water-sealed spirometer. The best value of 3 maneuvers was expressed as a percentage of the predicted value (% pred.,[7]).

The methacholine inhalation provocation concentration was measured by inhaling aerosols with increasing concentrations of the agent[8]. The solutions were nebulized with a Wiesbadener doppelspray (Wiesbadener Inhalatoren-Vertrieb, Wiesbaden, W-Germany) with an airflow of 8 $L.min^{-1}$. The output of the nebulizer is within 0.12 ± 0.02 $mL.min^{-1}$.

The aerosols were inhaled at tidal breathing, while the nose was clipped. The metha-

choline concentration ranged from 0.063 to 32 mg.mL^{-1}, concentration steps being 0.063, 0.125, 0.25, 0.50, 1, 2, 4, 8, 16 and 32 mg.mL^{-1} respectively. Each concentration was inhaled for 2 minutes at 5- minute intervals, until there was a fall in FEV_1 of 20% compared with the control inhalation (0.9% saline solution) or until the maximal concentration was inhaled. The provocation- concentration of methacholine, required to produce a fall in FEV_1 of 20% (PC_{20} -value) was calculated by interpolation of the logarithmic dose-response curve.

Symptom scores

At every visit to the clinic the patients were questioned about episodic wheezing and wheezing or chest tightness after exercise. These 2 complaints were scored semi quantitatively by the patient along a scale from 1 to 4 (1: severe complaints; 4: no complaints). The scores were added to form a general score (minimal=2, maximal=8).

Blood eosinophils and serum cortisol level

Before and after 8 wk of active treatment blood was sampled for the determination of the number of eosinophils (.mm^{-3}) and the cortisol level (nmol.L^{-1}). The blood samples were taken at the same time of the day for each patient.

Statistical analysis

Logarithmic transformation of the methacholine PC_{20} was used for statistical analysis. Values of FEV_1, PC_{20}, symptom scores, and serum cortisol level during active treatment were compared with values obtained during placebo treatment using Student's t-tests for paired observations. Changes in the number of eosinophils were evaluated using the sign test.

For each patient the effects of dosage and time on FEV_1, PC_{20}, and symptom scores were evaluated by multivariate analysis of variance (Manova,[9]). In addition, to assess the possible influence of a change in initial FEV_1 on the PC_{20}, an analysis of covariance was carried out. All the data are summarized as mean± S.E.M..

Results

Methacholine PC_{20}

The geometric mean value (±S.E.M.) of the methacholine PC_{20} for all patients before and after the placebo treatment period of 2 wk was 1.00 and 0.91 mg.mL^{-1}, respectively (ns). The mean values of the methacholine PC_{20} for both treatment groups are shown in Figure 1. The individual values are shown in Table 2.

In the low dosage group, methacholine PC_{20} increased from 0.90 to 1.21 and 1.55 mg.mL^{-1} after 2 and 8 wk of treatment, respectively (comparison with pretreatment value $p<0.05$ and $p<0.01$, respectively). In the high-dosage group, methacholine PC_{20} increased from 0.91 to 1.84 mg.mL^{-1} and 2.74 mg.mL^{-1} after 2 and 8 wk of treatment, respectively (both $p<0.001$).

During the whole study period, 800 µg/day budesonide induced a significantly higher increase of the methacholine PC_{20} values than the low dosage treatment (Manova,

Table 2. The effect of two different dosages of inhaled budesonide on methacholine PC_{20}.

		Budesonide 200 µg/day						Budesonide 800 µg/day			
Patient	before treatment	2wk	4wk	6wk	8wk	Patient	before treatment	2wk	4wk	6wk	8wk
1	0.25	0.34	0.73	0.93	0.53	16	2.33	13.93	12.02	6.92	15.28
2	0.40	1.08	0.06	1.22	0.74	17	1.56	1.32	2.52	2.09	2.43
3	2.13	3.48	2.62	2.66	2.78	18	9.68	11.48	13.46	22.96	29.65
4	0.98	1.43	0.56	0.71	0.69	19	0.13	0.21	0.33	0.33	0.36
5	4.00		4.17	6.38	6.28	20	1.04	1.63	2.46	2.93	2.98
6	0.87	1.56	2.52	1.97	2.46	21	0.10	0.44	0.24	0.33	0.33
7	3.35	10.72	3.89	4.05	5.37	22	2.97	3.85	8.69	3.84	3.79
8	0.21	0.46	0.44	0.78	0.50	23	2.93	4.00	2.97	3.36	3.54
9	0.95	0.82	0.62	0.62	0.35	24	0.79	3.48	3.06	1.66	3.94
10	0.44	1.55	2.83	1.15	2.49	25	0.89	2.04	3.12	5.01	7.59
11	1.56	0.79	1.52	3.62	2.00	26	0.36	1.04	0.96	0.62	0.87
12	0.60	0.36	0.44	0.33	0.65	27	0.72	1.95	1.74	2.46	5.71
13	1.35	3.16	4.59	3.40	4.59	28	1.21	2.87	1.45	1.52	2.29
14	0.40	0.72	0.66	1.68	1.42	29	1.24	1.25	1.91	1.77	1.43
15	1.81	1.26	0.97	1.87	2.64	30	0.21	0.38	0.15	0.64	1.42
Geometric mean	0.90	1.21*	1.10	1.56**	1.55**		0.91	1.84‡	1.89‡	1.99‡	2.74‡

Statistical significance with the pretreatment PC_{20} value:
* $p<0.05$ ** $p<0.01$ ‡ $p<0.001$

$p<0.001$). There was no significant (linear) time effect although the methacholine PC_{20} at 8 wk was signifantly higher than at 2 wk of treatment ($p<0.05$).

The 8 patients who had been receiving inhaled corticosteroids before the study did not behave different from the other patients.

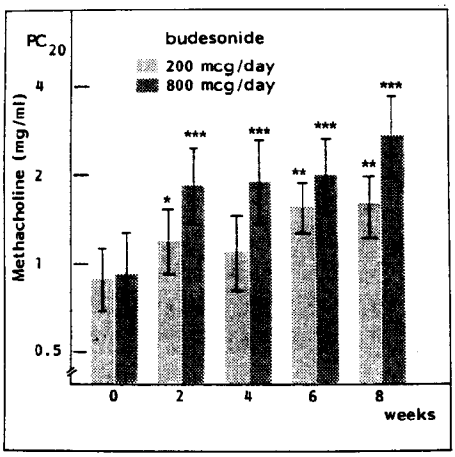

Figure 2. Effect of 2 different dosages of inhaled budesonide on methacholine PC_{20} (geometric mean values ± S.E.M.). Significant differences with the pretreatment value: * $p<0.05$ ** $p<0.01$ *** $p<0.001$.

Table 3. The effect of two different dosages of inhaled budesonide on percent predicted FEV_1.

		Budesonide 200 µg/day						Budesonide 800 µg/day			
Patient	before treatment	2wk	4wk	6wk	8wk	Patient	before treatment	2wk	4wk	6wk	8wk
1	99	108	108	101	100	16	100	99	95	104	103
2	92	102	95	97	105	17	93	100	11	93	100
3	99	116	110	110	116	18	80	78	77	80	76
4	85	82	82	87	87	19	82	93	89	89	89
5	94		97	98	102	20	89	98	101	103	104
6	85	90	95	87	91	21	87	101	89	99	85
7	116	116	110	123	119	22	82	92	92	90	87
8	72	84	81	85	79	23	83	82	91	96	87
9	78	101	101	109	91	24	72	86	88	87	92
10	81	87	84	85	85	25	94	96	98	96	94
11	106	96	100	103	106	26	90	106	105	100	106
12	81	85	92	85	88	27	78	91	88	84	86
13	108	108	109	107	103	28	78	84	81	77	86
14	78	85	84	84	84	29	94	104	97	93	97
15	88	88	86	84	82	30	66	82	87	87	82
Mean	91	96*	96*	96*	95*		84	93‡	93‡	93‡	92**
±SEM	3	3	3	3	3		3	2	2	2	2

Statistical significance with the pretreatment value:
* $p<0.05$ ** $p<0.01$ ‡ $p<0.001$

Lung funtion

The values of FEV_1 (% pred,± S.E.M.) are shown in Table 3 and Figure 3. In the low dosage group, FEV_1 increased by 4% ($p<0.05$) and in the high dosage group the FEV_1 increased by 8% ($p<0.005$) after 8 wk of treatment. There was a significant difference between the two dosages during the whole treatment period (Manova, $p<0.001$). A significant

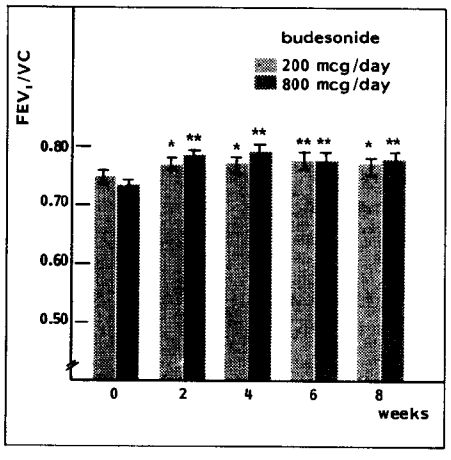

Figure 3. Effect of 2 different dosage of inhaled budesonide on the FEV_1 (mean values ± S.E.M.). Significant difference with the pretreatment value: * $p<0.05$ ** $p<0.01$.

improvement of FEV_1 was already reached after 2 wk of treatment. There was no further improvement in FEV_1 during the following 6 wk.

Symptom scores

There was no significant dosage effect on the symptom scores during the whole study period (Manova). After 2 wk of treatment, however, the patients in the high dosage group had fewer symptoms (p=0.06).

Eosinophil number in the peripheral blood and serum cortisol levels

As shown in Table 4, there was a significant decrease in the number of blood eosinophils in the high dosage group from 271± 48 to 206± 32.mm^{-3} ($p<0.05$, sign test). There was no decrease in the low dosage group. In neither of the two treatment groups a change in serum cortisol levels was observed. A significant correlation was observed between the log(methacholine PC_{20}) values and the blood eosinophil number (r=0.44, p=0.02).

Table 4. Blood eosinophils and serum cortisol level before and after 8 wk of treatment with inhaled budesonide.

	Blood eosinophils (.mm^{-3})		Serum Cortisol (nmol.l^{-1})	
	before treatment	after treatment	before treatment	after treatment
Budesonide, 200 µg/day	295 ± 65	292 ± 62	372 ± 47	369 ± 31
Budesonide, 800 µg/day	271 ± 48	206 ± 32 *	472 ± 48	448 ± 46

* Statistical significance from pretreatment value $p<0.05$ (sign-test).

Predictive value of initial methacholine PC_{20}, blood eosinophil number and serum IgE level for the treatment efect on the PC_{20}.

A multiple regression analysis of the change in PC_{20} methacholine as dependent variable with the treatment dosage, initial methacholine PC_{20}, initial blood eosinophil number, and serum IgE against house dust mite was carried out. Only the PC_{20} values before and after 8 wk of treatment were taken for analysis.

The model can be written as $\Delta\text{-}PC_{20} = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_4x_4$, where $\Delta\text{-}PC_{20}$ is the change in log(methacholine PC_{20}), a_0 is a constant, x_1, x_2, x_3, and x_4 are the dosage, log(initial PC_{20}), eosinophil number and IgE level against house dust mite respectively, and a_1, a_2, a_3, and a_4 are the corresponding regression coefficients.

The values calculated for a_0 to a_4 were $a_0 = 0.46\pm0.21$, $a_1 = 0.22\pm0.11$, $a_2 = -0.18\pm0.13$, $a_3 = 0.49\pm0.30$, and $a_4 = -0.15\pm0.98$.

Only the values for the constant a_0 and for a_1 were significantly different from zero, implying that apart from the dosage, no parameter was correlated to the change in PC_{20}.

Discussion

In the present study we demonstrated a dose-dependant effect of treatment with inhaled budesonide on methacholine PC_{20} in 30 allergic asthmatic patients. This effect was apparent at all stages of the 8 wk treatment period (Fig 1). We observed also a dose-dependent effect on FEV_1 but, because of the inclusion criteria of the patients (initial FEV_1 >70% of predicted value) the overall improvement of FEV_1 was limited. There was no significant dosage effect on the symptom scores. That no clear dosage effect on the symptom score was observed, may have been caused by the fact that the patients generally had only mild symptoms, which allowed us to withdraw the maintenance treatment before the study for at least 5 wk. During treatment, many patients became symptom free, while they still remained hyperresponsive to methacholine.

Although there was no significant linear time effect of budesonide on the methacholine PC_{20}, the lack of a linear time dependency of the change in PC_{20} does not preclude a significant time effect as indicated by the value after 8 wk of treatment being significantly higher than after 2 wk of treatment. The improvement in FEV_1 had reached its maximum already at 2 wk of treatment. No correlation was observed between the improvement in FEV_1 and in methacholine PC_{20}. These results suggest, that the corticosteroid effect on bronchial hyperresponsiveness is time dependent and that the improvement in bronchial hyperresponsiveness is not due to a change in airway caliber only.

Toogood and coworkers[10] demonstrated a dosage effect of inhaled corticosteroids on lung function and symptom scores in a group of patients with more severe asthma. A dosage effect on bronchial hyperresponsiveness, as we have observed in the present study, has not been shown before. Because we did not include a placebo-treated group inthe present study, we cannot give exact data on the difference between the effect of low dose budesonide compared with placebo. However, for 2 reasons, the presence of confounding effects of uncontrolled events that might change bronchial responsiveness is unlikely. In the first place, the methacholine PC_{20} of the patients remained quite stable during the placebo treatment period (14 days) before the active treatment. In the second place, the influence of an important confounding effect, altering exposure to important allergens, e.g., house dust mite, is unlikely because the study was carried out in the period between January and June. Furthermore, in the sea climate of the Netherlands, house dust mite sensitivity probably does not lead to a seasonal asthma.

With respect to the influence of the duration of treatment with corticosteroids, Ryan et al.[3], in their 4 wk treatment study with beclomethasone, found that the increase in histamine PC_{20} had already reached its maximum after 1 wk of treatment. There was no further improvement up to 4 wk of treatment. In our previous study[4], histamine PC_{20} tended to increase further after 4 wk, as compared to 2 wk of treatment.

Late allergic reactions are known to increase bronchial hyperresponsiveness[11] and it is therefore possible that bronchial hyperresponsiveness in allergic patients is, to some extent, determined by chronic exposure to allergens and ongoing late allergic reactions[12]. Treatment with corticosteroids prevents late allergic reactions[13,14] and the effect of these drugs on bronchial hyperresponsiveness is probably caused by a dampening of inflammatory processes occurring with ongoing allergic reactions.

In our patients a correlation was observed between the number of blood eosinophils

and the PC_{20} values which is in accordance with a recent report of Durham and Kay[15] who also found a correlation between these two parameters in allergic patients. In the present study we also found a significant decrease in the blood eosinophil number during treatment with the high dosage of budesonide (800 μg/day), whereas there was no decrease during treatment with the lower dosage. No changes in serum cortisol levels were seen after 8 wk of treatment with 800 μg budesonide/day. In our previous study with inhaled budesonide, we found a similar decrease in the number of blood eosinophils caused by treatment with 400 μg/day[4].

The importance of the eosinophil as an inflammatory cell in allergic reactions has been brought out in recent studies. Eosinophil infiltration in the lung[16] and increased number of blood eosinophils[17,18] are known to accompany late allergic reactions.

The decrease in blood eosinophils, that we observed during treatment with relatively low dosages of inhaled corticosteroids, may reflect a decrease in local production of eosinophil chemotactic factors in the lung.

Apart from the effect of the dosage on the change in bronchial hyperresponsiveness, the response of treatment varied largely interindividualy. Some patients had only a minor response, despite treatment with high dosage or a low initial methacholine PC_{20}. As it may be important to predict treatment response in individual patients we have attempted to find factors that may determine the treatment effect, i.e., the change in methacholine PC_{20}, apart from the dosage. We found no correlation between initial PC_{20}, eosinophilia or serum concentration of IgE against house dust mite with treatment-induced change in methacholine PC_{20}.

Although treatment with inhaled corticosteroids leads to a certain decrease in bronchial hyperresponsiveness, it also is apparent that the patients remain hyperresponsive during the treatment. An important question, not answered by our study, remains whether treatment with higher dosages of inhaled corticosteroids or a more prolonged treatment leads to a further decrease in bronchial hyperresponsiveness. If this kind of long term treatment should not lead to an important further improvement of hyperresponsiveness, than it becomes clear that the large part of this hyperresponsiveness in allergic patients is not determined by inflammatory processes and cannot be influenced by anti-allergic drugs.

The results of the present study lead to the conclusion, that the improvement in bronchial hyperresponsiveness in allergic asthmatic patients during treatment with inhaled corticosteroids is dosage dependant. The improvement in hyperresponsiveness is positively influenced by the duration of treatment. The decrease in the number of blood eosinophils that occurs during this treatment suggests that dampening of the allergic inflammatory process is one of the mechanisms by which bronchial hyperresponsiveness is decreased.

References

1. Boushey HA, Holtzmann M, Sheller JR, Nadel JA: Bronchial hyperreactivity. State of the Art. Am Rev Resp Dis 1980; 121:389-413.
2. Hargreave FE, Ryan G, Thomson NC, O'Byrne PM, Latimer K, Juniper EF, Dolovich J: Bronchial responsiveness to histamine or methacholine in Asthma: measurement and clinical significance J. Allergy Clin Immunol. 1981; 68:347-53.
3. Ryan G, Latimer KM, Juniper EF, Roberts RJ, Tech M, Hargreave FE. Effect of beclomethason dipropionate on bronchial responsiveness to histamine in controlled non-steroid-dependant asthma J Allergy Clin Immunol 1985; 75:25-30.
4. Kraan J, Koëter GH, v.d. Mark ThW, Sluiter HJ, de Vries K. Changes in bronchial hyperreactivity induced by 4 wk of treatment with antiasthmatic drugs in patients with allergic asthma: A comparison between budesonide and terbutaline. J Allergy Clin Immunol 1985; 76:628-36.
5. Kaliner M. Mechanisms of glucocorticoid action in bronchial asthma. J Allergy Clin Immunol 1985; 76; 321-9.
6. Johansson SA, Andersson KE, Brattsand R, Gruvstad E, Hedner P: Topical and systemic glucocorticoid potencies of budesonide, beclomethasone dipropionate and prednisolone in man. Eur J Respir Dis 1982; 63(suppl 122):74-82.
7. Tammeling GJ. Standard values for lung volume and ventilatorycapacity of sanatorium patients. Selected Papers. Royal Netherlands Tuberculosis Association 1961; 1:65.
8. De Vries K, Goei JT, Booy-Noord H, Orie NGM. Changes during 24 hours in the lung function and histamine hyperreactivity of the bronchial tree in asthmatic and bronchitic patients. Int Arch Allergy Appl Immunol 1962; 20:93-101.
9. Nie NH, Hull CH, Jenkins JG, Steinbrecher K, Bent DH. SPSS handbook, releases 7 to 9, Mcgraw Hill 1981.
10. Toogood JH, Lefcoe NM, Haines DSM, Jennings B, Errington N, Baksh L, Chuang L. A graded dose assessment of the efficacy of beclomethasone dipropionate aerosol for severe chronic asthma. J Allergy Clin Immunol 1977; 59:298-308.
11. Cockcroft DW, Ruffin RE, Frith PA, Hargreave FE et al. Determinants of allergen-induced asthma: dose of allergens circulating IgE antibody concentration and bronchial responsiveness to inhaled histamine. Am Rev Respir Dis 1979; 120:1053-8.
12. Kaliner M. Hypotheses on the contribution of the late-phase allergic response to the understanding of allergic diseases J Allergy Clin Immunol 1984; 73:311-6.
13. Booy-Noord H, Orie NGM, de Vries K. Immediate and late bronchialobstructive reaction to inhalation of house dust and protective effects of disodium cromoglycate and prednisolone. J Allergy Clin Immunol 1971; 48:344-54.
14. Cockcroft DW. Hypothesis: Mechanism of perennial allergic asthma. Lancet 1983;2: 253-6.
15. Durham SR, Kay AB. Eosinophils, bronchial hyperreactivity and late-phase asthmatic reactions. Clin Allergy 1985; 15:411-8.
16. De Monchy JGR, Kauffman H, Venge P, Koeter GH, Jansen HM, Sluiter HJ, de Vries K. Broncho-alveolar eosinophilia during allergen- induced late asthmatic reactions. Am Rev Respir Dis 1985; 131:373-8.
17. Booy-Noord H, de Vries K, Sluiter HJ,Orie NGM. Late bronchial obstructive reaction to experimental inhalation of house dust extract. Clin Allergy 1972; 2:43-61.
18. Dahl R, Venge P, Olssen I.Variations of blood eosinophil and eosinophilic cationic protein in serum in patients with bronchial asthma Allergy 1978; 33:211-5.

CHAPTER 10

CHANGES IN MAXIMUM EXPIRATORY FLOW-VOLUME CURVE CONFIGURATION AFTER TREATMENT WITH INHALED CORTICOSTEROIDS

J. Kraan, Th.W. van der Mark, G.H.Koeter.

Departments of Lung Diseases and Lung Function, Clinic of Internal Medicine, University of Groningen, the Netherlands.

Summary

The present study reports the changes in configuration of maximal flow-volume curves after 8 weeks treatment with inhaled corticosteroids, in 14 asthmatic patients. The configuration was compared with that seen after bronchodilation following inhalation of a single dose of ipratropium bromide. After inhaled corticosteroids the shape of flow-volume curves was less bowed towards the volume axis, whereas the shape of flow-volume curves after inhalation of ipratropium bromide showed no significant change. A significant correlation was observed between the decrease in blood eosinophil cell count and the straightening of the flow-volume curves, quantitatively expressed as shape factor and slope ratio. It is concluded that these changes in flow-volume curve configuration reflect a decrease in inhomogeneously distributed inflammatory airway narrowing.

Introduction

Bronchial obstruction in asthmatic patients is generally thought to be determined by bronchial smooth muscle contraction and by inflammatory processes in the bronchial wall[1]. Both factors can be treated separately. Inflammatory processes can be reduced by drugs such as corticosteroids, and smooth muscle contraction can be relieved by bronchodilating agents such as anticholinergic or sympathicomimetic drugs. Althought corticosteroids and bronchodilators act in a different way, both groups of drugs lead to an improvement in Forced Expiratory Volume in one second (FEV_1). Bronchodilation is achieved in a short time after administration of the bronchodilator, but anti-inflammatory treatment takes several weeks of therapy to be effective, owing to the nature of inflammatory pro-

Thorax 1989; 44:1015-21

cesses. In patients with mild asthma a rise in FEV_1 to their predicted values is often seen after administration of a bronchodilator, and such a change may occur after treatment with inhaled corticosteroids[2]. Other measures of pulmonary function could, however, show differences between the rapid bronchodilation seen with bronchodilator drugs and the bronchodilatation occuring over several weeks with anti-inflammatory treatment.

Maximal Expiratory Flow-Volume (MEFV) curves are generally thought to give additional information about the severity of bronchial obstruction[3,4]. The mechanical factors underlying airflow limitation during a forced expiration have received considerably more attention than the assessment of bronchial obstruction. On the basis of wave-speed mechanics, Dawson and Elliott[5] came to the conclusion that the flow-limiting segment moves towards the peripheral airways with decreasing remaining lung volume. Measuring flows at different lung volumes may therefore give an approximate indication of the site of airway obstruction.

In clinical practice the flow-volume curves are usually analyzed in terms of maximum flows at a given volume, and often interpreted qualitatively with regard to the shape of the curve. Even in patients with a mild bronchial obstruction[6,7] the flow-volume curves are more bowed towards the volume axis, and the question arises whether this increased convexity of the flow-volume curves reflects a specific pathological process that eventually may be influenced by treatment.

Several ways of quantifying the shape of an MEFV-curve have been put forward. Mead[9] developed the slope ratio (SR) defined as tangent slope $\dot{V}/dV$ divided by the chord $\dot{V}/V$ as an index of curvilinearity of the MEFV-curve. He introduced also the ratio 1/2 ($\dot{V}_{E,\,max\,50}/\dot{V}_{E,\,max\,25}$), here referred to as the shape factor at 50% remaining FVC (SF 50%), as an index of nonlinearity of the MEFV curve[10]. To extend the indices for curvilinearity of the MEFV-curves over a larger lung volume, we calculated a similar index, making use of the flow at 75% remaining FVC: $1/3(\dot{V}_{E,\,max\,75}/\dot{V}_{E,\,max\,25})$, the shape factor (SF) at 75% (fig.1).

In the present study MEFV curves were measured before and after administration of a single dose of ipratropium bromide, and before and after long-term treatment with budesonide, as part of another study[2]. Ipratropium bromide is a potent bronchodilator that blocks the bronchoconstricting effects of acetylcholine, released by the vagal nerve. Budesonide is a recently developed corticosteroid with a high local anti-inflammatory potency[8].

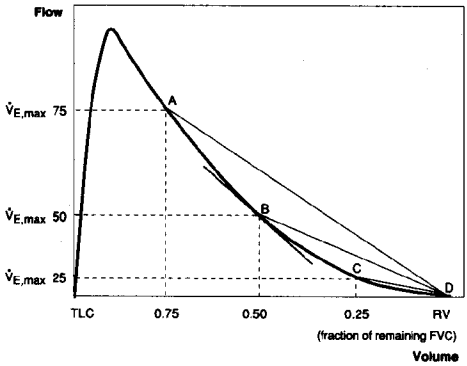

Figure 1. Schematic drawing of a curvilinear maximal expiratory flow volume (MEFV) curve, showing flows at 75%, 50%, and 25% remaining forced vital capacity (FVC) and the tangent at 50% FVC. Slope ratio at 50% FVC is defined as the slope of the tangent in point B, divided by the slope of the chord BD. Both shape factors and slope ratios are greater than 1 for a convex MEFV-curve. TLC: total lung capacity. RV: residual volume.

The MEFV-curves were analyzed in 14 patients with extrinsic asthma patients with a mild bronchial obstruction.

The aim of the study was to compare the effects of instantaneous bronchodilatation and bronchodilation resulting from long-term anti-inflammation treatment in terms both of the maximal expiratory flow at various lung volumes and of change of the shape of the MEFV-curve.

Methods

Patients

Fourteen patients (12 male and 2 female) with allergic asthma (mean age 26, range 18-36 years) gave their informed consent to participate in the study. The clinical characteristics of the patients are shown in Table 1.

Table 1. Lung function values (% predicted) at entry.

Patient	Age	FVC	FEV_1	PEFR	$\dot{V}_{E,max75}$	$\dot{V}_{E,max50}$	$\dot{V}_{E,max25}$
1	22	99	97	85	100	91	56
2	31	97	96	93	73	60	35
3	25	95	81	92	56	57	52
4	24	91	88	99	77	58	45
5	26	106	82	76	69	51	34
6	32	74	98	90	55	42	28
7	21	101	91	102	83	82	87
8	32	87	73	100	49	41	27
9	26	89	97	117	111	102	107
10	36	103	88	84	39	38	25
11	20	100	89	83	45	46	48
12	18	95	79	74	60	52	62
13	28	114	92	103	60	51	48
14	23	97	70	84	43	35	24
Mean	26	98	86	92	66	58	48
SEM	1	2	2	3	6	5	6

FVC: forced vital capacity; FEV_1: forced expiratory volume in one second; PEFR: peak expiratory flow rate; $\dot{V}_{E,max75}$, $\dot{V}_{E,max50}$, $\dot{V}_{E,max25}$: maximal expiratory flow rate at 75%, 50% and 25% remaining FVC respectively.

All patients had a history of episodic wheezing, and showed strongly positive skin test responses to at least two common allergens, including house dust mite (Allergens Diephuis laboratories, Groningen, the Netherlands). All had increased serum concentrations of IgE specific for house dust mite. All patients had increased bronchial hyperresponsiveness to inhaled methacholine (provocation concentration of methacholine causing a 20% decrease in FEV $PC_{20} < 8$ mg.mL^{-1}) according to the method of Juniper et al.[11], and had mild symptoms controlled by low doses of inhaled bronchodilators or prophylactic drugs (sodium cromoglycate or inhaled corticosteroids). None of the patients used oral

corticosteroids. The initial FEV_1, measured before the treatment period, was >70% of the predicted value[12].

Study design

Before entering the study, the patients discontinued their usual maintenance treatment (including sodium cromoglycate or inhaled corticosteriods) for at least 3 weeks. After this period the patients were treated for two weeks with placebo inhalations (single blind) followed by an active treatment period of 8 weeks, in which the patient used budesonide 800 μg/day by metered dose aerosol (2 puffs of 200 μg a puff, twice daily).

MEFV-curves were recorded before and after the active treatment period and blood was collected for eosinophil cell count. The drug cannisters were weighed every two weeks to assess the treatment compliance of the patients. Patients were allowed to use inhaled ipratropium bromide on an "if necessary" basis, to control symptoms throughout the study. No ipratropium bromide or budesonide was allowed for 12 hours preceding the visit to the clinic. In general, the patients had only mild symptoms and ipratropium bromide was taken infrequently.

At least 6 weeks after this active treatment period the patients came to the clinic to perform MEFV-curves before and 30 minutes after the inhalation of a single dosage of 80 μg ipratropium bromide (2 puffs containing 40 μg). The patients used only inhaled bronchodilator during the 3 weeks preceding this test and no inhaled drugs during the 12 hours preceding the test.

The protocol was approved by the hospital's medical ethics committee and written informed consent of each patient was obtained.

Lung function

MEFV-curves were recorded with a dry rolling-seal type spirometer (Mijnhardt, Vica test 5). After inspiring slowly to total lung capacity (TLC) the patient performed a forced expiration, followed by a forced inspiration. From three technically adequate curves where the forced vital capacity (FVC) did not differ by more than 5%, the curve with the largest $\dot{V}_{E,\,max\,50}$ was selected. Values of FVC, FEV_1, peak flow rate (PEFR), $\dot{V}_{E,\,max\,75}$, $\dot{V}_{E,\,max\,50}$, and $\dot{V}_{E,\,max\,25}$ were expressed as percentages of the predicted values[12]. The rate constant (RC) was calculated as $\dot{V}_{E,\,max\,50}/FVC$.

Curvature parameters were determined in three ways (fig. 1). The index of shape as introduced by Mead et al.[10] was defined as $1/2*(\dot{V}_{E,\,max\,50}/\dot{V}_{E,\,max\,25})$ (SF 50%). We extended the volume range by also calculating a similar index $1/3*(\dot{V}_{E,\,max\,75}/\dot{V}_{E,\,max\,25})$ (SF 75%). Mead 's slope ratio (SR) is defined as the tangent slope to the curve dV/dV divided by the chord $\dot{V}/V$[9].

As the microcomputer incorporated in the spirometer does not provide values for the slope of the MEFV-curves, we estimated the slope as follows. A quadratic curve was fitted to flows at 75%, 50%, 25% and 0% of the FVC, to derive the slope ratio at these points. These values were compared with those obtained graphically, estimated directly from the tracings. The values from the quadratic fit matched the values drawn by hand well. We estimated the error in the slope values to be less than 3 %. From values of $\dot{V}_{E,\,max\,75}$ and $\dot{V}_{E,\,max\,50}$, computed by spirometer, we calculated the chords, and hence the slope ratios at 75% and 50% of the remaining FVC.

Analysis

We calculated the intra-individual standard deviation from 2 consecutive measurements on the same day, using one-way analysis of variance. The intraindividual SD for the shape factor was 0.08 in absolute units (5%) at 75% and 0.08 (6%) at 50%. The intraindividual SD for the slope ratios at 75% was 0.17 in absolute units (12%) at 75 % and 0.21 (16%) at 50 %.

Statistical analysis

Student's t-test for paired samples was used to compare posttreatment with baseline values of MEFV curve values and curvature parameters. Correlations between variables were calculated by the Rank-Spearman test.

Results

Changes in lung function with budesonide and ipratropium are summarized in Table 2. There were no significant differences between the baseline values before the two treatments, indicating that six weeks after cessation of budesonide, the MEFV-curve had returned to its pretreatment shape.

Table 2. Maximal Expiratory Flow Volume values before and after treatment.

	Budesonide		Ipratropium bromide	
	baseline	after treatment	baseline	after treatment
FVC	98 (2)	103 (2)**	101 (2)	104 (2)‡
FEV_1	86 (2)	96 (2)‡	86 (3)	95 (2)‡
PEFR	91 (3)	108 (4)*	95 (4)	108 (4)‡
$\dot{V}_{E,max75}$	66 (6)	80 (4)*	67 (7)	82 (6)‡
$\dot{V}_{E,max50}$	58 (5)	70 (4)**	59 (5)	72 (6)‡
$\dot{V}_{E,max25}$	48 (6)	69 (5)‡	51 (6)	63 (6)‡

Mean values (sample SEM) are expressed as percentage predicted. ‡: $p < 0.001$; **: $p < 0.005$; *: $p < 0.01$. Significant differences are assessed using paired t-test. Abbreviations as in Table 1.

Budesonide treatment for 8 weeks caused a significant improvement in all lung function measurements. The percentage change in end expiratory flows, especially in VE,max,25 (59%) was much greater than the change in FEV_1 (12%). Administration of ipratropium bromide also caused a significant improvement in expiratory volumes and flows. The change in $\dot{V}_{E,max25}$ (28%), was less pronounced in relation to change in FEV_1 (11%) than after treatment with budesonide.

The change in curvilinearity of the MEFV curve (shape factor and slope ratio at 75% and 50% FVC) is summarized in Table 3, and individual changes are given in fig. 2 and

Table 3. Measures (mean (SEM)) of the shape of the MEFV-curve.

	Budesonide		Ipratropium bromide	
	baseline	after treatment	baseline	after treatment
RC	0.64 (0.06)	0.74‡ (0.05)	0.64 (0.06)	0.77‡ (0.07)
SF 50%	1.36 (0.09)	1.12* (0.07)	1.29 (0.07)	1.27ns (0.06)
SF 75%	1.59 (0.13)	1.30** (0.11)	1.49 (0.09)	1.46ns (0.09)
SR 50%	1.33 (0.06)	1.26ns (0.06)	1.32 (0.06)	1.31ns (0.05)
SR 75%	1.43 (0.07)	1.30* (0.08)	1.41 (0.07)	1.40ns (0.06)

RC: Rate constant: $V_{E,max50}$/FVC (unit: sec-1); SF:shape factor at 50% FVC and 75% FVC respectively; SR: slope ratio at 50% FVC and 75% FVC respectively. *: $p < 0.025$; **: $p < 0.01$; ‡: $p < 0.001$; ns: not significant.

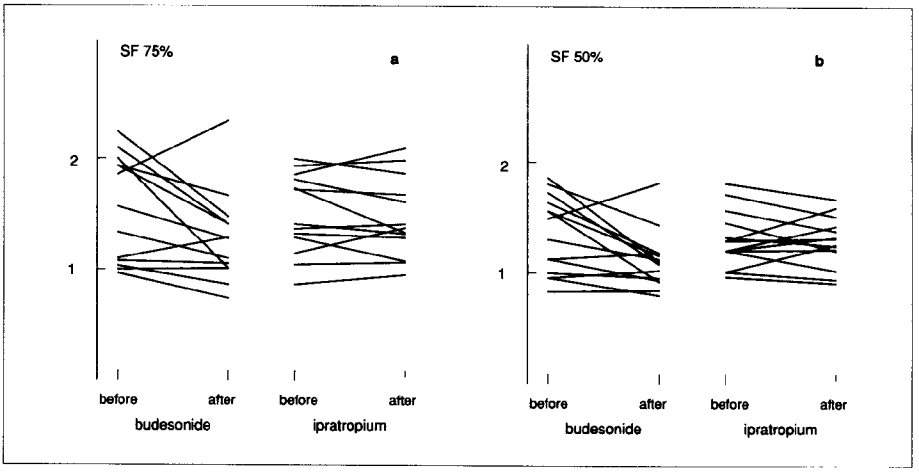

Figure 2. Individual changes in shape factor at 75% remaining FVC (SF 75%) (a), and at 50% remaining FVC (SF 50%) (b), before and after the treatment with budesonide and ipratropium bromide.

fig. 3. At 75% FVC both slope ratio and shape factor had fallen significantly after treatment with budesonide; ipratropium bromide caused no change in either measure. At 50% FVC only the shape factor showed a significant decrease with budesonide. Changes in shape factor were larger when the initial slope factor was higher (see figs 2 and 3), indi-

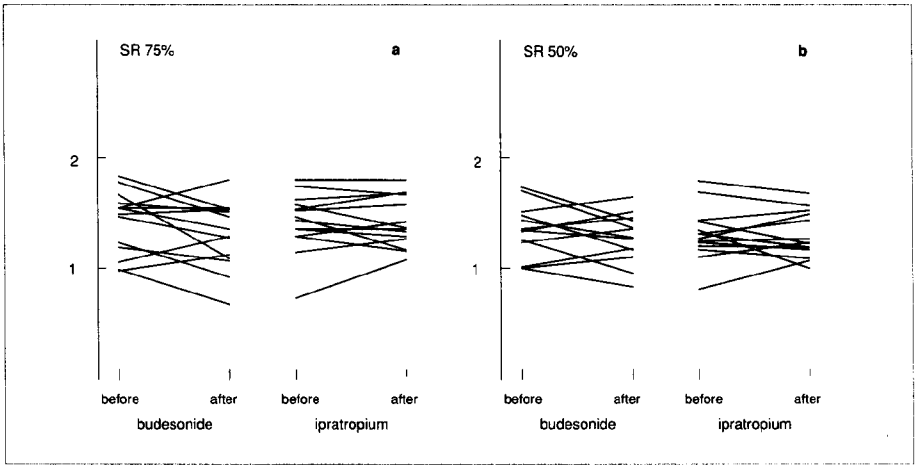

Figure 3. Individual changes in slope ratio at 75% remaining FVC (SR 75%) (a), and at 50% remaining FVC (SR 50%) (b), before and after the treatment with budesonide and ipratropium bromide.

cating that the more convex to the volume axis the MEVF curve is the larger is the improvement with budesonide.

Slope ratios decreased significantly with decreasing lung volume, for both baseline measurements and after administration of ipratropium bromide. After budesonide slope ratios were generally smaller, and showed no relation to lung volume (fig. 4.).

The mean (SEM) number of eosinophil granulocytes in peripheral blood decreased significantly from 267 (51).mm^{-3} to 198 (33).mm^{-3} after treatment with budesonide ($p < 0.05$, non-parametric sign test). There was a correlation between the ratio of blood eosinophils before and after treatment and the change in the shape factor with budesonide (Spearman correlation coefficient 0.47, $p < 0.05$, fig. 5).

Discussion

In this study treatment with both budesonide for eight weeks and a single dose of ipratropium bromide increase FEV_1. Budesonide had a larger effect on the end-expiratory maximal flows than ipratropium bromide. This may partly be due to the fact that the end-expiratory maximal flow values are numerically smaller and show wide variability. As the variability for these flows appears to be in the same order of magnitude as for other maximal expiratory flow values in our patients (Table 2), the differences we report are likely to reflect difference in action of budesonide and ipratropium bromide.

The end expiratory flow rates are frequently used to assess bronchodilator responses[13,14]. The present study shows that possible changes in curvilinearity of the MEFV curve also need to be taken into account. In this study we quantified the curvilinearity of the MEFV curves and showed that treatment with budesonide led to a decrease in curvilinearity while treatment with ipratropium bromide had no such an effect. The straightening

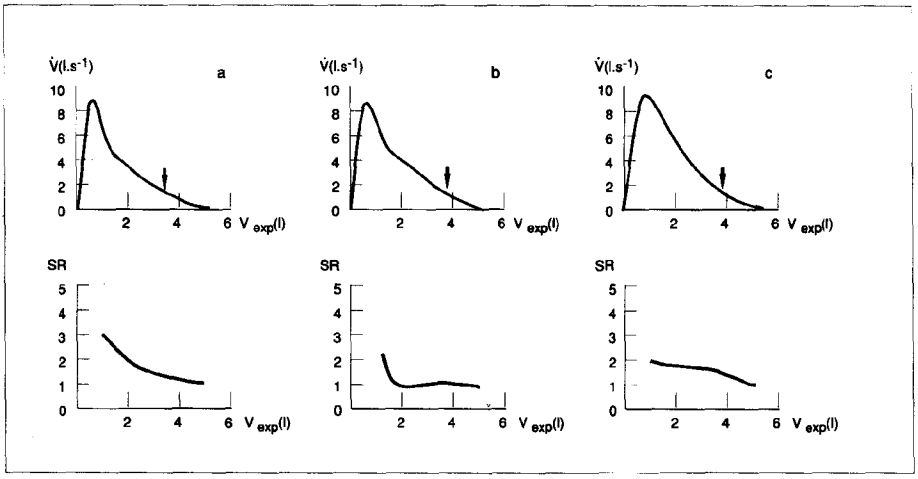

Figure 4. Example of MEFV-curves of a single patient. a: baseline curve; b: curve after 8 weeks treatment with budesonide; c: curve after 6 weeks withdrawal of budesonide, but after inhalation of ipratropium bromide. Arrows indicate FEV_1. In the lower panel the course of the slope ratio versus volume (V) is shown.

effect of budesonide on the MEFV curve was seen with all three methods of quantifying the curvilinearity, although the methods that used the values of $\dot{V}_{E,\,max\,75}$ were more sensitive. The slope ratio at 50% FVC showed a non significant difference, whereas the change in slope ratio at 75% FVC was significant. Before treatment with budesonide there appears to be a decrease of the slope ratio with decreasing remaining lung volume. This confirms the findings of O'Donnell[15] and coworkers, who observed such a decrease in subjects with mild asthma. They also found no such change in slope ratio in normal subjects. The fact that we found no significant difference in the slope ratio's at 75% and 50%

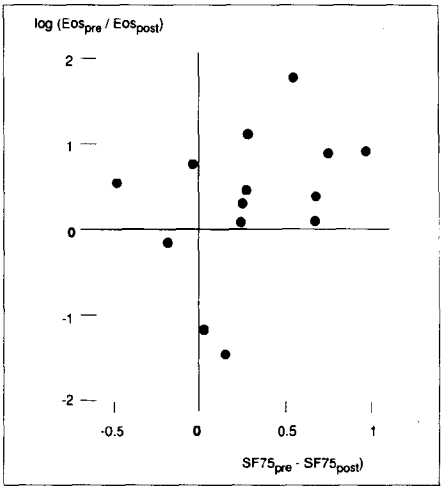

Figure 5. Scatterplot of the logarithm of the ratio of the eosinophil cell counts (Eos) and the difference in shape factor (SF 75), both before and after the treatment with budesonide.

FVC after treatment with inhaled corticosteroids might indicate that the change in the slope ratio with decreasing lung volume has returned to normal. It may be argued that selection of the MEFV curves introduce bias with respect to the curvilinearity measures. Peslin et al.[16] reviewed different methods of selection of MEFV curves, and found that the method of selection resulted in systematic changes in the maximal expiratory flow values. The absolute values of the curvilinearity parameters may therefore depend on our selection criteria, but differences in these parameters before and after medication are unlikely to be influenced by the selection, similarly, though measurements by dry rolling seal spirometers may differ from those made by pneumotachography[17], these differences are systematic, and cancel out when values before and after medication are compared.

The shape factor seems to be a fairly repeatable parameter, as judged by intra-individual standard deviations. Slope ratio was more variable, perhaps because slope ratio is calculated from differentiation towards volume. We made no corrections for change in absolute lung volume. Neither treatment would be expected to cause much change in absolute lung volume in these patients with mild asthma (Table 2). As both shape factor and slope ratio are basically ratios of flows, which shift in the same direction with change lung volume, the effect of a small change in lung volume on flow would be expected to cancel out in the ratios of flows, and probably therefore had only a minor effect upon shape factor and slope ratio.

The pathophysiological basis of the increased curvilinearity of the MEFV-curve is unknown. Many authors are of the opinion that the decrease of the expiratory flow near residual volume is due to preferential obstruction of the peripheral airways[18,19]. Mead[9] developed the concept of inhomogeneous emptying of the lung during a forced expiration and has shown on theoretical grounds that when such inhomogeniety occurs the flow-volume curve should be convex towards the volume axis. This has also been seen by O'Donnell[15] in asthmatic subjects, by Landau[20] in patients with more advanced obstructive lung disease, and recently by Kapp et al.[21] in an epidemiological study. The increased curvilinearity of the MEFV-curve may therefore be caused by regional inhomogeneity of forced expiratory flow, -that is the existence of regions with a different time constant (flow divided by volume) in the lung[9]. Our results might therefore suggest that inhaled corticosteroid drugs improve regional ventilatory inhomogeneity whereas rapid bronchodilation did not exert such an effect, although the overall time constant was decreased.

Bronchial obstruction in asthma seems to be the consequence of smooth muscle contraction and increased thickness of the airway wall caused by inflammatory processes[1]. These inflammatory changes are seen in central as well as in peripheral airways[22]. The eosinophil cell, which is predominant in bronchial biopsy material in allergic subjects[22] and in broncho-alveolar lavage fluid[23] from asthmatic subjects, is seen in samples taken from central airways[22,23]. The eosinophil may be a marker of disease activity as suggested by Durham and Kay[24], who observed that the number of blood eosinophils correlated well with the degree in BHR.

Budesonide has a strong anti-inflammatory effect[8] and this is thought to account for the fall in the number of peripheral blood eosinophils during treatment with budesonide. Since budesonide has a low systemical bioavailability when inhaled[8], we assume that the decrease in peripheral blood eosinophils is due to inhaled and not to ingested drug. The fact that we found a correlation between the change in peripheral blood eosinophils and

the change in the curvilinearity of the MEFV-curve suggests that inflammatory processes might be responsible for the abnormal shape of MEFV-curves in asthmatic patients. Limitation of expiratory flow is related not only to airway narrowing but also to the mechanical properties of the airway wall, in particular to airway compliance[5]. Therefore, thickening of the airway walls due to inflammation, may be an important cause of flow-limitation. Such changes in the thickness of the airway wall are unlikely to be spread uniformly throughout the airways, and this may lead to inhomogeneous emptying during forced expiration.

Ipratropium bromide is a powerful bronchodilator. Several studies have shown that regional ventilation inhomogeneity in asthma is not improved by administration of a bronchodilator[25,26]. This might be due to better penetration of the aerosol to relatively well ventilated areas in contrast to relatively poor ventilated areas. The effect of long-term treatment with an inhaled corticosteroid drug might differ from the effect of the inhaled bronchodilator bwcause the former causes a gradual decrease in airway narrowing in poorly ventilated areas, thereby decreasing ventilation inhomogeneity.

The question whether the anti-inflammatory effect occurs preferentially in central or in peripheral airways cannot be answered from this study. The detection of inhomogeneous emptying of the lung during a forced expiration is determined by the resistance that different lung regions have in common. If this common resistance is large in relation to the resistances peripheral to the common resistance, the total time constant would be primarily determined by the common resistance. From the results reported here, it may be concluded that, before anti-inflammatory treatment, the major inhomogeneity is located peripherally, whereas after treatment the flow-limiting segment has moved to more central sites. This does not, however, rule out the possibility that central airways are also affected by inflammation. In that case the main effect of treatment with inhaled corticosteroids would be a change in central airway compliance.

We conclude that treatment with an inhaled corticosteroid causes an increase of maximal expiratory flows and a decrease of the abnormal curvilinearity of the MEFV-curve in patients with allergic asthma. This effect is probably due to a decrease in inhomogeneously distributed inflammatory airway narrowing. This reduction in curvilinearity of the MEFV- curve was not seen after bronchodilation by a single dose of ipratropium bromide, despite a similar increase in FEV_1.

References

1. Dunnill MS. The pathology of asthma with special reference to the changes in the bronchial mucosa. J Clin Pathol 1960; 13:27-33.

2. Kraan J, Koeter GH, Van der Mark ThW, Boorsma M, Kukler J, Sluiter HJ, de Vries K. Dosage and time effects of inhaled budesonide in bronchial hyperreactivity. Am Rev Resp Dis 1988; 137:44-48.

3. Hyatt RE, Black LF. The flow-volume curve. A current perspective.Am Rev Resp Dis 1973; 107:191-9.

4. Olive JT, Hyatt RE. Maximal expiratory flow and total respiratory resistance during induced bronchoconstriction in asthmatic subjects. Am Rev Resp Dis 1972; 106:366-76.

5. Dawson SV, Elliott EA. Wave-speed limitation on expiratory flow - a unifying concept. J Appl Physiol 1977; 43:498-515.

6. Black LF, Offord K, Hyatt RE. Variability in the maximum expiratory flow-volume curve in asymptomatic smokers and in non-smokers. Am Rev Resp Dis 1974; 110 :282-92.

7. Green M, Mead J, Turner JM. Variability of maximum expiratory flow-volume curves. J Appl Physiol 1974; 37:67-74.

8. Johansson SA, Andersson KE, Brattsand R, Gruvstad E, Hedner P. Topical and systemic glucocorticoid potencies of budesonide, beclomethasone diproprionate, and prednisolone in man. Eur J Respir Dis 1982; 63 (suppl. 122):74-82.

9. Mead J. Analysis of the configuration of maximum expiratory flow-volume curves. J Appl Physiol 1978; 44:156-65.

10. Hyatt RE, Rodarte JR, Mead J, Wilson ThA. Changes in lung mechanics; Flow-Volume relation In: The lung in the transition between health and disease, Macklem PT, Permutt S (eds), Marcel Dekker, New York.

11. Juniper EF, Frith PA, Dunnett C, Cockroft DW, Hargreave FE. Reproducibility and comparison of responses to inhaled histamine and methacholine. Thorax 1978; 33:705-10.

12. Quanjer PhH. (ed). Standardized lung function testing. Clin Resp Physiol 1983; 19 (suppl. 5):45-51.

13. Cohen AA, Hale FC. Comparative effects of isoproterenol aerosols on airway resistance in obstructive pulmonary diseases. Am J Med Sci 1965; 249:309-15.

14. Clay MM, Pavia D, Clarke SW. Effect of aerosol particle size on bronchodilatation with nebulised terbutaline in asthmatic subjects. Thorax 1986; 41:364-8.

15. O'Donnell CR, Castile RG, Mead J. Changes in flow-volume curve configuration with bronchoconstriction and bronchodilation. J Appl Physiol 1986; 61:2243-51.

16. Peslin R, Bokadana A, Hannhart B, Jardin P. Comparison of various methods for reading maximal expiratory flow-volume curves. Am Rev Resp Dis 1979; 119; 271-8.

17. Pincock AC, Miller MR. The effect of Temperature on Recording Spirograms. Am Rev Resp Dis 1983; 128:894-8.

18. Knudson RJ, Burrows B, Lebowitz MD. The maximal expiratory flow volume curve: its use in the detection of ventilatory abnormalities in population study. Am Rev Resp Dis 1976; 114:871-79.

19. Gelb AF, Zamel N. Simplified diagnosis of small airway obstruction. N Eng J Med 1973; 288:395-8.

20. Landau LI, Taussig LM, Macklem PT, Beaudry PH. Contribution of inhomogeniety of lung units to the MEFVC in children with asthma and cystic fibrosis. Am Rev Resp Dis 1975; 111:725-31.

21. Kapp MC,Schachter EN,Beck GJ, Maunder LR, Witek ThJ. The Shape of the Maximum Expiratory Flow-Volume Curve. Chest 1988; 94:799-806.

22. Holgate ST, Roche W, Roberts A, Beasley RC. Inflammation as the basis of asthma. In: Bronchitis IV, fourth international symposium, Sluiter HJ, van der Lende R, Gerritsen J, Postma DS (eds), van Gorcum Assen 1989.

23. De Monchy JGR, Kauffman HF, Venge P, Koeter GH, Jansen HM, Sluiter HJ, de Vries K. Broncho-alveolar eosinophilia during allergen-induced late asthmatic reactions. Am Rev Resp Dis 1985; 131:373-6.

24. Durham SR, Kay AB. Eosinophils, bronchial hyperreactivity and late-phase asthmatic reactions. Clin Allergy 1985; 15:411-8.

25. McFadden ER, Lyons HA. Airway resistance and uneven ventilation in bronchial asthma. J.Appl Physiol 1968; 25:365-70.

26. Siegler D, Fukuch Y, Engel L. Influence of bronchomotor tone on ventilation distribution and airway closure in asymptomatic asthma. Am Rev Resp Dis 1976; 114:123-30.

SUMMARY AND CONCLUSIONS

Many asthmatic patients experience shortness of breath or wheezing, when exposed to cold air, or irritants like baking fumes, exhaust gases or cigarette smoke. This clinical phenomenon has been called bronchial hyperresponsiveness (BHR), which is defined as an exaggerated broncho-obstructive response following exposure to a small quantity of a non-allergic stimulus that does not provoke such a response in normal subjects. BHR has been observed in patients with asthma, emphysema, and bronchitis, and it is therefore regarded as a characteristic phenomenon in chronic obstructive lung diseases.

The underlying mechanism of BHR, although extensively studied, is only partly understood. Possible mechanisms include an imbalance in autonomic nervous control, intrinsic hyperresponsiveness of airway smooth muscle or mediator releasing cells, and airway inflammation (e.g. caused by viral infection, exposure to chemical irritants and allergic reactions).

The degree of BHR has been shown to be related to the severity of the disease and to the amount of drugs required to control symptoms. Furthermore, it has been suggested that the degree of BHR is correlated to the rate of annual decline in lung function in chronic airflow obstruction. For these reasons, reduction of BHR can be considered as an important aim in the treatment of patients with obstructive lung diseases, and the effectiveness of anti-asthmatic drugs in this respect might be an important criterion of their usefulness.

This thesis deals with the effect of anti-asthmatic drugs on bronchial hyperresponsiveness. The pharmacokinetic behaviour of some of these drugs has been studied. These studies will be summarized first, followed by a summary of the studies on the effect of drugs on BHR.

The pharmacokinetic behaviour of xanthine derivatives in disease

The bronchoprotective effect of xanthines is probably dependent on adequate plasma drug concentrations. Unfortunately, theophylline has a rather narrow therapeutic range, and too high plasma concentrations may lead to serious side effects. Therefore it is necessary to keep a certain margin of safety to avoid side effects. Theophylline is mainly eliminated by biotransformation in the liver. Its disposition is more dependent on the intrinsic biotransformation capacity of liver cells than on hepatic blood flow. The pharmacokinetic behaviour of theophylline shows large interindividual variation, and is influenced by many factors such as age, drug interactions, disease states such as liver cirrhosis and congestive heart failure. Therefore, effective treatment with theophylline is only possible when plasma concentrations are monitored (sometimes frequently), especially in conditions such as congestive heart failure, respiratory insufficiency or severe pneumonia.

The recently developed xanthine derivative enprofylline has a different pharmacoki-

netic profile, being excreted, mainly unmetabolized, by the kidney with a short plasma half-life. Its clearance has been reported to be correlated to the creatinine clearance, which might make it, at least to some extent, predictable if information about renal function is available. If, moreover, the elimination rate of enprofylline during acute disease states was shown to be more stable than that of theophylline, treatment with enprofylline could be expected to be safer than with theophylline, especially in acute disease states.

In this thesis the pharmacokinetic behaviour of theophylline and enprofylline was studied in patients with liver cirrhosis, patients with chronic renal failure, and in patients with an acute exacerbation of chronic airflow obstruction, whether or not complicated by hypoxaemia or heart failure. In addition, the pharmacokinetics of these drugs are studied in an experimental intravenous dosing model.

In Chapter 4 the pharmacokinetic behaviour of theophylline and enprofylline was examined in patients with an acute exacerbation of chronic obstructive lung disease (COLD). The study was carried out in a group of eight patients with uncomplicated COLD, and a group of ten patients with COLD complicated by hypoxaemia or congestive heart failure. The elimination constant (K_{el}) was determined in the acute phase and during recovery after 6 days of treatment. During continuous treatment in the intermediate period the total body clearance of the xanthine was determined daily.

There was a large interindividual variation in elimination for both drugs, especially in the patient group with complicated COLD. In the acute phase, the elimination of both theophylline and enprofylline was decreased considerably in the patients with COLD complicated by hypoxaemia or congestive heart failure, compared with the patient group with uncomplicated COLD. However, there was a partial recovery of the elimination parameters during clinical improvement of the patients. The clearance of enprofylline was significantly correlated to the creatinine clearance.

The decreased theophylline elimination in the acute phase observed in the patients with complicated COLD probably reflects impairment of the intrinsic biotransformation capacity of the liver, which is known to occur in congestive heart failure and probably also in cor pulmonale. The decreased elimination of enprofylline in the patients with congestive heart failure or hypoxaemia coincided with a decreased creatinine clearance. It may have been caused by decreased renal blood flow. It is also possible that hypoxaemia impairs active excretion of enprofylline.

In Chapter 5, the pharmacokinetic behaviour of theophylline and enprofylline in patients with liver cirrhosis, patients with chronic renal failure, and healthy control subjects is described and compared. In addition, the predictive value of routine tests of liver function and renal function (creatinine clearance) for the theophylline and enprofylline total body clearances is assessed.

The plasma clearance of theophylline, which is eliminated by biotransformation in the liver, was significantly decreased in patients with liver cirrhosis compared with both patients with renal failure and control subjects. The plasma clearance of enprofylline, which is mainly excreted unmetabolized by the kidney, was significantly decreased in patients with chronic renal failure, compared with both patients with liver cirrhosis and control subjects. A strong correlation was observed between creatinine clearance and

enprofylline clearance, while there was only a weak correlation between the liver function tests and theophylline clearance.

In Chapter 6, a method of infusing drugs with an exponentially decreasing delivery rate was described. This infusion method has previously been recommended as a tool for rapidly and safely attaining effective and stable plasma concentrations. For drugs whose distribution and elimination can be described by a simple one-compartment model, a stable plasma level can be instantaneously attained by administering an intravenous bolus dose, which raises the plasma concentration to the desired level, followed by an infusion given at the same rate as the elimination rate. For drugs that also distribute to a more peripheral or deeper compartment, the situation is more complex. The regimen proposed above will lead to a more or less prominent overshoot in plasma concentration, and the time for attainment of the stable plasma concentration will depend on the late elimination half-life. However for these drugs, which obey two-compartment pharmacokinetics, a plateau plasma level can be instantaneously attained if the initial bolus dose, which raises the concentration in the central compartment to the desired level, is followed by an infusion that compensates for both the distribution to the peripheral compartment and the elimination. Thus while the plasma concentration of the drug remains stable, the drug concentration in the peripheral compartment rises gradually. The speed of this rise may differ between various tissue compartments.

In the present study we used the infusion method with an exponentially decreasing delivery rate for the administration of two xanthine derivatives, theophylline and enprofylline, and we investigated whether it was feasible to create consecutive plasma concentration plateaus within a few hours. The infusions were carried out on two separate study days in 8 subjects with stable asthma. Before the actual infusion experiments, the pharmacokinetics of the substances in the individual subjects were determined on a separate study day.

The study showed that plasma concentration rose to the desired level already within 5 minutes after the start of the infusion of each subsequent dose, and that a stable plasma concentration plateau was maintained during the following 90 minutes of infusion. It was possible to achieve 4 subsequent concentration plateaus within a period of 6 hours. The use of the infusion method resulted in predictable plasma concentrations at all levels, and the method thus appears safe when the required plasma concentrations are below the toxic level.

Conclusions of the pharmacokinetic studies:

1. In patients with liver cirrhosis, theophylline clearance may be decreased, as has been reported by previous studies. This might lead to potentially toxic plasma concentrations when a routine dosage scheme is used. Enprofylline elimination is not reduced, at least when renal function is not impaired in these patients. In contrast, in patients with chronic renal disease, enprofylline clearance may be severely diminished, depending on the degree of renal impairment. In these patients theophylline clearance is not decreased.

2. As was previously found for theophylline, enprofylline clearance may be severely diminished in acute exacerbations of COLD, when these exacerbations are complicated by clinical conditions such as heart failure or hypoxaemic pulmonary conditions. When the clinical condition improves, enprofylline elimination increases as well. The observed temporary decrease in enprofylline clearance coincides with a decrease in creatinine clearance, and may be caused by a decrease in renal blood flow or in active renal excretion in the acute phase.

3. There was a good correlation between creatinine clearance and enprofylline clearance in patients and healthy subjects, while there was only a weak correlation between some routine liver function tests and theophylline clearance. The predictive value of the creatinine clearance for the enprofylline clearance is reasonably good, although there remains a rather large unexplained interindividual variance. Routine liver function tests have no predictive value for theophylline clearance.

4. Effective and safe treatment with theophylline and enprofylline should aim at plasma concentration ranges of 8-16 $mg.L^{-1}$ and 2-4 $mg.L^{-1}$ respectively. When information about renal function is available and when enprofylline dosing is adapted to renal function, toxic plasma drug concentrations may be more easily avoided with enprofylline than with theophylline. However, although it may be easier to give rough guidelines about initial treatment (e.g. on basis of serum creatinine values) for enprofylline than for theophylline, it is our opinion, that plasma concentrations should be frequently measured during maintenance treatment with both xanthine drugs, in view of the many factors that influence drug clearance especially in acute illness. It is also our opinion that patients with severely disturbed liver or renal function should preferably be treated with the drug that is expected to have the shortest expected plasma half-life.

5. A simple infusion method with an exponentially decreasing delivery rate can be used to obtain instantaneously steady state drug plasma concentrations. The infusion procedure can be repeated to create consecutively increasing plasma concentration plateaus. The method is feasible for drugs with a short as well as drugs with a prolonged plasma half-life. Apart from clinical situations in which effective drug dosages are to be administered rapidly, the method appears to be useful in pharmacologic dose-response studies.

The effect of anti-asthmatic drugs on bronchial hyperresponsiveness

The effect of drugs on BHR can be immediate, occurring within minutes after administration and lasting for some hours, or sustained, after repeated administration of the drug. Alternatively, there may be a long-term effect on BHR, occurring only after maintenance treatment for weeks or possibly months.

After acute administration of bronchodilators, such as beta-agonists and xanthine derivatives, a sometimes pronounced decreasing effect on BHR is observed. This effect is generally of short duration, but it can be sustained by repeated administration or by giving

the drug in a sustained release preparation. The inhibitory effect of beta-agonists and xanthines on methacholine and histamine responsiveness is probably caused by functional antagonism. Although it is generally thought that bronchodilators have no permanent influence on the pathophysiology of BHR, little is known about the effects on BHR of long-term treatment (e.g. after weeks or months of treatment) with these drugs. Prolonged treatment with beta-adrenergic drugs may lead to beta-receptor desensitization, as has been demonstrated in several target tissues. Whether this effect might lead to a change in bronchial responsiveness has been subject of our studies.

If inflammatory processes are an important causative factor in BHR, it would be expected that anti-inflammatory drugs, such as corticosteroids, have a decreasing effect on BHR, although it might be necessary to administer these drugs for a prolonged period of time before the beneficial effect could be observed. In this thesis, the effects on BHR of some bronchodilatory agents (terbutaline and the xanthine derivatives theophylline and enprofylline) and an anti-inflammatory drug (budesonide) have been studied.

In chapter 7, the effect of increasing intravenous doses of theophylline and enprofylline on bronchial hyperresponsiveness to methacholine has been examined. Eight young patients with allergy participated in the study.

Methacholine provocations were carried out on three separate days, using a double-blind study design, before and after increasing doses of theophylline, enprofylline, and placebo. Methacholine responsiveness was measured as the provocation concentration causing a fall of 20% in FEV_1 compared with baseline (PC_{20}). The patients had been characterized pharmacokinetically on a separate occasion. An individual dosage scheme was designed for each patient in order to attain 3 consecutive instantaneous steady-state plasma concentration plateaus at desired levels of 5, 10, and 15 $mg.L^{-1}$ for theophylline and 1.25, 2.5, and 3.75 $mg.L^{-1}$ for enprofylline, using the infusion method described in Chapter 6. After a baseline methacholine provocation, the next 3 challenges were carried out under protection of these 3, progressively increasing, plasma drug concentrations or placebo.

There was a small progressive decrease in FEV_1 following repeated methacholine challenges during placebo. Both drugs caused a significant dose-related increase in FEV_1 values compared with placebo. The geometric mean (methacholine) PC_{20} value decreased significantly following placebo, due to the repeated (methacholine) challenges. PC_{20} values were significantly higher after theophylline and enprofylline than after placebo, the maximum difference being 2.0 and 1.7 methacholine doubling dose, respectively. The overall effect of both drugs on methacholine PC_{20} was dose-related. The increase in methacholine PC_{20} was directly correlated to the bronchodilatory effect (both effects measured as changes compared with placebo), which is in accordance with the assumption that the protective effect of xanthines is caused by functional antagonism. There was already a significant protective effect at the lowest plasma concentrations of both drugs (theophylline 5.3 $mg.L^{-1}$; enprofylline 1.2 $mg.L^{-1}$), while the rise of the plasma concentrations to the highest level (15.0 and 3.7 $mg.L^{-1}$ respectively) did not lead to an important increase of the effect.

Chapter 8 describes a double-blind crossover study comparing the effects of long-term treatment of inhaled budesonide and terbutaline on bronchial hyperresponsiveness

in 17 patients with allergic asthma. Both drugs were given for 4 weeks, with a placebo-treatment period before and after each active treatment period. To assess bronchial hyperresponsiveness, inhalation provocation tests with histamine and propranolol were performed every 2 weeks. Before each inhalation provocation, the drugs were withheld for at least 12 hours.

Before the budesonide treatment the FEV_1 value (% predicted) was 85.3 ± 4.1% (mean ± SEM). After 2 and 4 weeks of treatment with this drug, this value increased significantly to 89.4 ± 4.1 and 96.2 ± 3.8, respectively. The histamine provocation concentrations causing a decrease in FEV_1 of 20% (PC_{20}) on the same days were 4.0, 7.2 and 9.5 $mg.mL^{-1}$, respectively. The PC_{20} values for propranolol, which were measured 1 hour after the histamine provocation, were 11.7, 13.3 and 14.0 $mg.mL^{-1}$. respectively.

The FEV_1 values before and after 2 and 4 weeks of treatment with terbutaline were 86.2 ± 4.0, 84.8 ± 4.1 and 87.0 ± 4.6%, respectively. The histamine PC_{20} values on the same days were 4.7, 3.1 and 3.8 $mg.mL^{-1}$ respectively. The propranolol PC_{20} values were 14.2, 8.7 and 10.1 $mg.mL^{-1}$. This study demonstrated opposite effects of budesonide and terbutaline on BHR. Maintenance treatment with budesonide led to a progressive improvement in BHR after 4 weeks, while treatment with terbutaline led to an increase in BR, which appeared to subside after 4 weeks of treatment. While the effect of budesonide is probably caused by a dampening of late allergic reactions, the long-term effect of terbutaline may be caused by beta-receptor desensitization.

Chapter 9 investigates the possibility whether treatment with inhaled budesonide has a dose- and time-dependent effect on the degree of bronchial hyperresponsiveness. A double-blind study was carried out in 2 parallel groups, each containing 15 allergic asthmatic patients. The patients were randomly allocated to treatment with either 200 or 800 µg budesonide per day for a period of 8 wk. The active treatment period was preceded by a selection period of 3 wk, and a single-blind placebo period of 2 wk. During these initial 5 wk, the maintenance treatment of the patients, including disodium chromoglycate and inhaled corticosteroids, was withheld. Spirometry and inhalation provocation tests with methacholine were carried out and the symptom score was recorded every 2 wk. The methacholine provocation concentrations (geometric mean) causing a decrease in FEV_1 of 20% (PC_{20}) in the 200 µg/day and 800 µg/day treatment groups just before the active treatment period were 0.90 and 0.91 $mg.mL^{-1}$ respectively. These values increased significantly to 1.21 and 1.84 $mg.mL^{-1}$ after 2 wk of treatment, and to 1.55 and 2.74 $mg.mL^{-1}$ after 8 wk of treatment. During the whole study period, budesonide in a dosage of 800 µg/day induced a significantly larger change in PC_{20} than in a dosage of 200 µg/day. The FEV_1 before treatment was 91 ±3% (SEM) and 84 ±2% of the predicted values in the two groups. The FEV_1 values after 2 wk of treatment were 96 ±3% and 93 ±2% in the 200 µg/day and 800 µg/day treatment groups respectively. There was no further improvement in FEV_1 during the following 6 wk of treatment, in contrast to the PC_{20} values.

Treatment with budesonide led to a decrease in the number of eosinophil cells in the peripheral blood, while there was no decrease in serum cortisol concentration, suggesting a dampening influence on the allergic inflammatory process in the airways. In individual subjects,the initial degree in BHR, initial blood eosinophil count, or serum IgE concentration, were of no predictive value for the response to treatment, measured as the change in methacholine PC_{20} from baseline.

The study demonstrated that inhaled budesonide can diminish bronchial hyperresponsiveness in allergic asthmatic patients significantly, and that this change is dose-dependent. Furthermore, the results indicate that the improvement in bronchial hyperresponsiveness is influenced in a positive way by the duration of treatment. Although FEV_1 is a relatively insensitive parameter of airway diameter, the lack of correlation between the observed changes in BHR and FEV_1 suggests that a reduction of airway wall thickening cannot be the sole explanation for the observed improvement in BHR caused by corticosteroids.

Chapter 10 reports on the changes in configuration of maximal expiratory flow-volume curves (MEFV-curves) after 8 weeks of treatment with inhaled corticosteroids in 14 asthmatic patients. These configurations are compared with those after instantaneous bronchodilation, obtained after inhalation of a single dose of ipratropium bromide.

Even in patients with mild bronchial obstruction, the MEFV-curve is convex towards the volume axis. The exact pathophysiological basis of increased curvilinearity of the MEFV-curve is unknown. It may be caused by preferential obstruction of peripheral airways or by regional inhomogeneity of forced expiratory flow. This abnormality seems to be a very sensitive parameter of mild bronchial obstruction.

Several ways of quantifying the shape of an MEFV-curve have been put forward. Mead developed the slope ratio (SR), defined as tangent slope ($\dot{V}/dV$) divided by the chord $\dot{V}/(V\text{-}FVC)$, as an index of curvilinearity of the MEFV-curve. He introduced also the ratio 1/2 ($\dot{V}_{E,\,max\,50}/\dot{V}_{E,\,max\,25}$) as an index of nonlinearity of the MEFV-curve and called it the Shape Factor at 50% remaining FVC (SF 50%). To extend the indices for curvilinearity of the MEFV-curves over a larger lung volume, we calculated a similar index, making use of the flow at 75% remaining FVC: $1/3(\dot{V}_{E,\,max\,75}/\dot{V}_{E,\,max\,25})$, the Shape Factor (SF) at 75%.

We found that after treatment with inhaled corticosteroids, the flow-volume curves were less convex towards to the volume axis, whereas the MEFV-curves after inhalation of ipratropium bromide showed no significant changes. Ipratropium bromide is a powerful bronchodilator. Several studies have shown that regional ventilation inhomogeneity in asthma is not improved by administration of a bronchodilator. This might be caused by better penetration of the aerosol in relatively well ventilated areas, in contrast to relatively poorly ventilated areas. The long-term effect of an inhaled corticosteroid drug might differ from the effect of the inhaled bronchodilator in the sense that the former causes a gradual decrease in airway narrowing in poorly ventilated areas, thereby decreasing ventilation inhomogeneity. The reported effects may reflect a decrease in inhomogeneously distributed inflammatory airway narrowing.

The question whether the anti-inflammatory effect occurs preferentially in central or in peripheral airways cannot be answered from this study. Inhomogeneous emptying of the lung during a forced expiration can be detected from the resistance that different lung regions have in common. If this common resistance is large compared with the resistances preceding the common resistance, the total time constant would be primarily determined by the common resistance. From the results reported here, it may be concluded that before anti-inflammatory treatment, the major inhomogeneity is located peripherally, whereas after treatment the flow-limiting segment has moved to more cen-

tral sites. However, this does not rule out the possibility that central airways are also affected by inflammation.

Conclusions of the studies on effects of drugs on bronchial hyperresponsiveness

1. The protection provided by the xanthine derivatives theohylline and enprofylline against methacholine hyperresponsiveness is dose-dependent. The increase in methacholine PC_{20} is directly correlated to the bronchodilatory effect. The maximal protective effect on methacholine PC_{20} is about 2 doubling doses. There is, however, already a significant protective effect at relatively low plasma xanthine concentrations, while the rise of plasma concentrations to the maximal attainable level does not lead to an important increase of the effect. These results may imply that in clinical practice it is not necessary to achieve the highest possible xanthine plasma concentration to obtain an optimal protective effect against bronchoconstrictive stimuli.

2. Maintenance treatment with beta-agonists may lead to increased BHR as demonstrated by the decrease in histamine and propranolol PC_{20}, measured 12h after cessation of treatment with inhaled terbutaline. This change in BR is probably due to desensitization of beta-receptors on airway smooth muscle or cholinergic airway ganglia.

This increase in BHR appears to be small and also seems to be transient, because in our study the increased responsiveness to histamine and propranolol had disappeared when treatment with terbutaline was continued for 4 weeks. However, an increase in BHR induced by beta-agonist therapy may be important when asthma has worsened as a result of a viral infection or an allergic reaction, both factors that may increase BHR (and have a decreasing effect on beta-receptor function), which in turn may lead to a tendency to over-use beta-agonists. Such a coincidence of factors that have the potency to increase BHR may be relevant to the pathophysiology of a severe asthmatic attack.

3. Our studies with budesonide demonstrated that maintenance treatment with inhaled corticosteroids, leads to a decrease in BHR in asthmatic subjects. This effect is not dependent on a coincident improvement in FEV_1. The decrease in BHR is dose-dependent. In individual subjects, factors like initial degree in BHR, blood eosinophil number, and serum IgE concentration do not predict the response to treatment measured as the change in methacholine PC_{20} from baseline. This might imply that corticosteroids can be expected to improve BHR in allergic as well as non-allergic patients. The decrease in blood eosinophil count following treatment with inhaled budesonide suggests that corticosteroids inhibit the eosinophil attraction to the lungs which occurs in the course of allergic airway inflammatory processes.

4. In general, even in mild asthmatic subjects with a normal FEV_1, there is an abnormal configuration of the maximal expiratory flow-volume curve, which is convex towards to the volume axis. Quantitative expression of the configuration of the MEFV-curves in Shape Factor and Slope Ratio, showed that after treatment with inhaled corticosteroids, the flow-volume curves were less convex, whereas the MEFV-curves after inhalation of

ipratropium bromide showed no significant changes. The reported effects may reflect a decrease in inhomogeneously distributed inflammatory airway narrowing.

5. Although the studies on the effect of corticosteroids on BHR confirm the already existing evidence that inflammatory processes form an important causative factor for BHR, treatment with a relatively high dosage of budesonide, even for 8 weeks, has a modestly improving effect on BHR. The question to what extend inflammatory processes contribute to the cause of BHR, might be answered by further studies on the effect of more prolonged maintenance treatment with inhaled corticosteroids.

Future research on the effect of anti-asthmatic drugs on BHR will probably give answers to questions that are important for clinical practice, but also to questions about the mechanisms of BHR and the importance of BHR as a central phenomenon in obstructive lung diseases.

SAMENVATTING EN CONCLUSIES

Patiënten met astma ervaren na blootstelling aan koude lucht, baklucht of rook, veelal wisselende kortademigheid gepaard gaand met piepen op de borst. Een dergelijke versterkte reactie, in de vorm van luchtwegvernauwing, na blootstelling aan kleine hoeveelheden niet-allergene prikkels die bij normalen geen reactie veroorzaken, wordt bronchiale hyperreactiviteit (BHR) genoemd.

BHR wordt waargenomen bij patiënten met astma, emfyseem en chronische bronchitis en wordt daarom als een kenmerkend verschijnsel van chronische aspecifieke respiratoire aandoeningen (CARA) beschouwd. Het oorzakelijk mechanisme van BHR is, ondanks uitgebreid onderzoek, slechts ten dele bekend. Factoren die wellicht een rol spelen zijn een stoornis in de regulatie van het autonome zenuwstelsel, dat ingrijpt op het gladde spierweefsel rond de bronchiën, toegenomen reactiviteit van het gladde spierweefsel en toegenomen reactiviteit van cellen in het bronchiale weefsel die mediatoren, stoffen die contractie van glad spierweefsel veroorzaken, vrijmaken. Daarnaast wordt waarschijnlijk ook een belangrijke rol gespeeld door ontstekingsprocessen in het bronchiale slijmvlies, veroorzaakt door allergische reacties, virale infecties of blootstelling aan chemische irritantia.

Er zijn aanwijzingen dat de ernst van de BHR verband houdt met de prognose van CARA. Er blijkt een verband te bestaan tussen de mate van BHR, de ernst van de CARA en de uitgebreidheid van de medicamenteuze behandeling die nodig is om de klachten te bestrijden. Om deze reden kan het doen afnemen van de BHR beschouwd worden als een belangrijk doel in de behandeling van patiënten met CARA. Een belangrijk criterium voor de toepasbaarheid van medicamenten wordt dan ook gevormd door de mate waarin ze de BHR doen verminderen.

In dit proefschrift werd onderzocht welk effect medicamenten die bij de behandeling van CARA worden gebruikt hebben op de BHR. Daarnaast werden de pharmacokinetische eigenschappen van enkele van deze medicamenten onderzocht. Hieronder worden eerst de resultaten van deze studies samengevat, gevolgd door de samenvatting van het onderzoek naar de effecten van medicamenten op de BHR.

De invloed van ziekteprocessen op de pharmacokinetiek van xanthine derivaten

Het effect van xanthine derivaten op BHR is waarschijnlijk afhankelijk van een adequate plasma spiegel van het medicament. Te hoge plasma concentraties echter, kunnen ernstige toxische effecten tot gevolg hebben. Om deze reden is het noodzakelijk bij de dosering een veilige marge in acht te nemen om bijwerkingen te voorkomen. theofylline wordt voornamelijk geëlimineerd in de lever en vertoont grote interindividuele verschillen in de eliminatiesnelheid, welke o.a. afhankelijk is van leeftijd, medicament interacties en ziekten zoals leverziekten en hartinsufficiëntie. Frequente meting van plasma concentraties is soms noodzakelijk voor een effectieve en veilige behandeling met theofylline, vooral in acute situaties zoals respiratoire insufficiëntie en hartinsufficiëntie.

Het recent ontwikkelde xanthine derivaat enprofylline heeft van theofylline afwijkende pharmacokinetische eigenschappen. Het wordt grotendeels ongemetaboliseerd uitgescheiden door de nieren met een korte plasma halfwaarde tijd. De eliminatie van enprofylline is gecorreleerd aan de nierfunctie, uitgedrukt in de creatinineklaring. Deze eigenschap maakt het mogelijk, indien gegevens over de nierfunctie bekend zijn, het pharmacokinetisch gedrag van enprofylline te voorspellen. Als de eliminatie van enprofylline tijdens acute ziekte minder aan variatie onderhevig zou blijken te zijn dan de eliminatie van theofylline, zou dit middel met name tijdens acute situaties toegepast kunnen worden.

In dit proefschrift werd de pharmacokinetiek van theofylline en enprofylline in patiënten met levercirrose en patiënten met chronische nierinsufficiëntie, alsmede in CARA-patiënten met een acute verergering van hun luchtwegvernauwing (bij een luchtweginfectie) al dan niet gecompliceerd door respiratoire insufficiëntie of hartinsufficiëntie.

De pharmacokinetiek van deze beide medicamenten werd ook onderzocht in een experimenteel intraveneus toedieningsmodel.

In hoofdstuk 4 werd de pharmacokinetiek van theofylline en enprofylline onderzocht in CARA-patiënten met een acuut verergerde luchtwegvernauwing. Het onderzoek werd uitgevoerd bij patiënten met luchtwegvernauwing al dan niet gecompliceerd door ademhalings- of hartinsufficiëntie.

Voor beide medicamenten was er een grote interindividuele variatie van de eliminatiesnelheid. Bij de patiënten met ademhalings- of hartinsufficiëntie was tijdens de acute fase, de eliminatie van theofylline en enprofylline sterk verminderd. Tijdens de herstelfase nam de eliminatiesnelheid van theofylline en enprofylline weer toe. De eliminatiesnelheid van enprofylline was significant gecorreleerd aan de nierfunctie (creatinineklaring).

In hoofdstuk 5 werd de pharmacokinetiek van theofylline en enprofylline in patiënten met levercirrose en patiënten met chronische nierinsufficiëntie en een groep gezonde proefpersonen beschreven en vergeleken. Bovendien werd de voorspellende waarde van routine laboratoriumbepalingen van lever- en nierfunctieparameters voor de eliminatiesnelheid van theofylline en enprofylline onderzocht.

De plasmaklaring van theofylline dat geëlimineerd wordt door biotransformatie in de lever, was significant verminderd in de patiënten met levercirrose terwijl de plasmaklaring van enprofylline verminderd was in patiënten met chronische nierinsufficiëntie. Er bleek een goede correlatie tussen de enprofyllineklaring en de creatinineklaring te bestaan, terwijl er een geringe correlatie bestond tussen de leverfunctietests en de theophyllineklaring.

In hoofdstuk 6 werd een intraveneus infuussysteem met een exponentieel afnemende toedieningssnelheid beschreven. Deze infusiemethode die al eerder beschreven werd, kan gebruikt worden om op snelle en veilige wijze effectieve en stabiele plasmaconcentraties van een medicament te bereiken.

De infusiemethode werd gebruikt om theofylline en enprofylline intraveneus toe te dienen. Nagegaan werd of het mogelijk was met deze infusiemethode zodanig te doseren dat binnen enkele uren stapsgewijs opklimmende en stabiele plasmaconcentratie niveaus bereikt konden worden. De infusie van beide medicamenten werd op afzon-

derlijke dagen toegediend aan patiënten met een stabiele bronchusobstructie. Bij voorafgaand onderzoek waren van de afzonderlijke medicamenten in ieder individu pharmacokinetische gegevens, zoals verdelingsvolume en eliminatie constantes gemeten om het doseringsschema bij de eigenlijke infusie-studies te kunnen vaststellen.

Nadat de plasmaconcentratie binnen 5 minuten tot het gewenste niveau was verhoogd bleek het mogelijk te zijn dit niveau vervolgens stabiel te handhaven. Op deze wijze konden binnen een periode van 6 uur 4 stapsgewijs opklimmende plasma concentratie niveaus bereikt en gehandhaafd worden. Het gebruik van deze infusie methode resulteerde in voorspelbare plasma concentratie op alle niveaus.

Conclusies van de pharmacokinetische studies

1. Bij patiënten met levercirrose kan, zoals uit eerdere publicaties is gebleken, de theophyllineklaring aanzienlijk verminderd zijn. Dit kan leiden tot potentieel toxische plasma concentraties wanneer een routine doseringsschema wordt gebruikt. De enprofyllineklaring is in deze patiënten, als de nierfunctie niet gestoord is, niet verminderd.

2. Zoals bekend was voor theofylline, kan ook de eliminatie van enprofylline bij CARA-patiënten sterk verminderd zijn tijdens een acute verergering van luchtwegvernauwing door ademhalings- of hartinsufficiëntie. Als de klinische toestand verbetert, treedt ook veelal weer een stijging van de eliminatiesnelheid op. De waargenomen vermindering van de enprofyllineklaring valt samen met een tijdelijk verminderde nierfunctie.

3. Er bestond een goede correlatie tussen de enprofyllineklaring en de creatinineklaring in alle patiënten groepen, terwijl theophyllineklaring slechts zwak gecorreleerd was met de resultaten van routine leverfunctietests. De voorspellende waarde van de creatinineklaring voor de enprofyllineklaring is goed, terwijl de leverfunctietests geen voorspellende waarde voor de theophyllineklaring hebben.

4. Voor een effectieve en veilige behandeling met theofylline en enprofylline moeten plasmaconcentraties tussen respectievelijk 8-16 $mg.L^{-1}$ en 2-4 $mg.L^{-1}$ nagestreefd worden. Toxische plasmaconcentraties van enprofylline kunnen waarschijnlijk gemakkelijker vermeden worden dan van theofylline, wanneer gegevens beschikbaar zijn over de nierfunctie en wanneer de enprofylline dosering aangepast wordt aan de nierfunctie.

Hoewel het waarschijnlijk gemakkelijker is grove richtlijnen aan te geven voor de aanvankelijke dosering van enprofylline (bijv. met behulp van serum creatinine waarden) is het ook ten aanzien van dit medicament wenselijk dat frequent plasmaconcentraties gemeten worden, vooral tijdens acute ziektebeelden. Het is bovendien aan te bevelen patiënten met ernstig gestoorde lever- of nierfunctie te behandelen met het medicament met de (te verwachten) kortste halfwaardetijd.

5. Een infuussysteem waarmee medicamenten met exponentieel afnemende snelheid worden toegediend, blijkt goed bruikbaar te zijn om een gewenste stijging van de

plasmaconcentratie van het medicament vrijwel momentaan te bereiken en stabiel te handhaven. De infusieprocedure kan herhaald worden teneinde trapsgewijs opklimmende plasmaconcentratie niveaus te bereiken. De methode is bruikbaar voor medicamenten met een korte halfwaardetijd en kan zowel klinisch als in pharmacologisch onderzoek toegepast worden.

Het effect van medicamenten op bronchiale hyperreactiviteit

Na toediening van een bronchusverwijdend medicament, zoals een beta-agonist of een xanthine derivaat kan een, soms sterke, vermindering in de mate van BHR optreden. Dit effect is in het algemeen kortdurend, maar het kan verlengd worden door het medicament herhaald toe te dienen. Het effect van deze medicijnen op de BHR op methacholine en histamine berust waarschijnlijk op functioneel antagonisme. Hoewel men in het algemeen van mening is dat bronchusverwijdende medicamenten geen blijvend effect op de mate van BHR hebben, is er weinig bekend over het effect van langdurige behandeling (bijv. weken of maanden) op de BHR. Langdurige behandeling met beta-agonisten kan leiden tot beta-receptor desensitisatie, zoals werd aangetoond in verscheidene weefsels. Of dit effect kan leiden tot een vermindering in de mate van BHR is onderzocht in dit proefschrift.

Als chronische ontstekingsprocessen van belang zijn als oorzakelijke factor van BHR, mag verwacht worden dat ontstekingsremmende medicijnen, zoals corticosteroiden, de mate van BHR kunnen doen verminderen. Het mag echter verwacht worden dat dit effect pas optreedt nadat deze medicamenten voor langere tijd als onderhoudsbehandeling zijn gebruikt. In dit proefschrift zijn de effecten van enkele luchtwegverwijdende medicamenten (de beta-agonist terbutaline en xanthine derivaten theofylline en enprofylline) en een ontstekingsremmend middel (het corticosteroid budesonide) bestudeerd.

In hoofdstuk 7 is het effect van stapsgewijs opklimmende intraveneuze doseringen van theofylline en enprofylline op de methacholine BHR onderzocht. Het onderzoek werd uitgevoerd bij jonge allergische CARA-patiënten met een reversibele luchtwegobstructie.

De methacholine inhalatie-provocaties werden uitgevoerd op drie afzonderlijke dagen, voor en na opklimmende doseringen van theofylline, enprofylline en placebo. De BHR op methacholine werd gemeten als provocatie concentratie die een 20 % daling van het geforceerde uitademingsvolume in een seconde (FEV_1) ten opzichte van de uitgangswaarde (PC_{20}) veroorzaakt.

Beide medicamenten veroorzaakten, vergeleken met placebo, een significant dosis-afhankelijke toename in de FEV_1.

De gemiddelde PC_{20}-waarden onder invoed van theofylline en enprofylline waren significant hoger dan tijdens placebo. Het effect van beide medicamenten op de methacholine PC_{20} was dosis afhankelijk

Het maximale beschermende effect tegen de methacholine inhalatie provocatie bedroeg ongeveer een verviervoudiging van de methacholine PC_{20}. Beide medicamenten verschilden niet in hun beschermend effect bij de gebruikte doseringen. De toename in methacholine PC_{20} was direct gecorreleerd aan de verbetering in longfunctie, hetgeen in overeen-

stemming is met de veronderstelling dat het effect van deze medicamenten berust op een functioneel antagonisme. Een gering protectief effect was reeds meetbaar bij de laagste plasmaconcentraties van beide medicamenten (theofylline 5.3 $mg.L^{-1}$; enprofylline 1.2$mg.L^{-1}$) terwijl er niet een belangrijke toename van het beschermende effect optrad als de plasma concentratie werd verhoogd naar het hoogste niveau (respectievelijk 15.0 $mg.L^{-1}$ en 3.7 $mg.L^{-1}$).

In hoofdstuk 8 wordt het effect van langdurige behandeling met het inhalatie corticosteroid budesonide vergeleken met het effect van behandeling met de beta-agonist terbutaline op de BHR bij allergische CARA-patiënten. Beide medicijnen werden gedurende 4 weken als onderhoudsbehandeling gegeven in een cross-over studie, terwijl de actieve behandelingsperioden voorafgegaan en gevolgd werden door een placebo-periode. De mate van BHR werd gemeten door histamine en propranolol inhalatieprovocaties.

Onder invloed van onderhoudsbehandeling met budesonide nam de BHR geleidelijk af terwijl onderhoudsbehandeling met terbutaline een tijdelijke toename van BHR veroorzaakte. Bovendien verbeterde budesonide de longfunctie (FEV_1 en piekstroomsnelheid) en deed het de dagschommeling in piekstroomwaarden en de astmatische klachten afnemen. Uit deze studie blijkt dat budesonide en terbutaline een tegengesteld effect op de BHR hebben. Het effect van budesonide wordt mogelijk veroorzaakt door een demping van late allergische reacties terwijl het effect van terbutaline mogelijk veroorzaakt wordt door beta-receptor desensitisatie.

In hoofdstuk 9 werd de mogelijkheid van een dosis- en tijdsafhankelijk effect op de BHR door een langdurige inhalatie-therapie met budesonide onderzocht. Allergische CARA-patiënten werden behandeld met 200 µg of 800 µg budesonide per dag gedurende een periode van 8 weken. Het bleek dat het effect van budesonide dosis-afhankelijk was. Met 800 µg/dag budesonide werd op ieder tijdstip een grotere verbetering van de BHR bereikt dan met 200 µg/dag. Het effect na 8 weken behandeling was groter dan na 2 weken behandeling. Individuele parameters als aanvankelijke mate van BHR, serum IgE gehalte en initieel aantal eosinofiele cellen in het perifere bloed hadden geen voorspellende waarde voor de individuele respons op de therapie.

Hoofdstuk 10 beschrijft de veranderingen in de vorm van de maximale expiratoire flow-volume (MEFV) curve van allergische CARA-patiënten na 8 weken behandeling met het inhalatie corticosteroid budesonide.

MEVF-curves geven aanvullende informatie over de mate en ernst van luchtwegvernauwing. Naast kwantitatieve informatie, de maximaal mogelijke uitademingssnelheid bij een bepaald longvolume, geeft de vorm van de MEFV-curve aanvullende informatie over de mate van luchtwegvernauwing. Reeds bij patiënten met geringe luchtwegvernauwing vertoont de flow-volume curve een convexe kromming in de richting van de volume-as. Hoewel de oorzaak van dit fenomeen niet precies bekend is, kan het berusten op het feit dat luchtwegvernauwing niet gelijkmatig over de luchtwegen verspreid is. De kromming van de MEFV-curve die optrad na 8 weken behandeling met budesonide werd vergeleken met de verandering na het luchtwegverwijdende medicament ipratropiumbromide.

Na behandeling met het inhalatiecorticosteroid bleek de kromming van de MEFV-curve af te nemen, terwijl er na ipratropium inhalatie geen verandering in de vorm van de curve optrad.

Conclusies van de studies over de effecten van de medicamenten op de bronchiale hyperreactiviteit

1. Het protectieve effect van de xanthine derivaten theofylline en enprofylline tegen de BHR op methacholine is dosis afhankelijk. Het effect op de BHR is direct gecorreleerd aan het luchtwegverwijdende effect. Uit het verloop van de methacholine PC_{20} in afhankelijkheid van de xanthine plasma concentratie mag geconcludeerd worden dat het niet altijd noodzakelijk is de hoogst mogelijk plasma concentraties te bereiken voor een optimaal protectief effect.

2. Onderhoudsbehandeling met beta-agonisten kan leiden tot toegenomen BHR. Deze toename in BHR is mogelijk het gevolg van desensitisatie van beta-receptoren van het bronchiale gladde spierweefsel of op parasympatische ganglia. Dit effect lijkt van tijdelijke aard te zijn, aangezien het niet meer aantoonbaar was na 4 weken onderhoudsbehandeling.

Een toename in BHR, geinduceerd door behandeling met beta-agonisten kan klinische van belang zijn als de CARA is verslechterd door een allergische reactie of virale infectie (beide factoren kunnen de BHR doen toenemen), hetgeen weer kan leiden tot overgebruik van beta-agonisten.

3. Zelfs in patiënten met een lichte CARA heeft de MEFV-curve veelal een afwijkende vorm. In een onderzoek waarbij deze vormafwijking gekwantificeerd werd, bleek dat na behandeling met inhalatiecorticosteroiden deze vervorming afnam, terwijl eenmalige inhalatie van een bronchusverwijdend middel geen verandering in de MEFV-curve teweegbracht.

De waargenomen effecten weerspiegelen mogelijk een gelijkmatiger verdeling en verbetering van de luchtwegweerstand in de luchtwegen.

4. Behandeling met inhalatiecorticosteroiden, zoals budesonide, leidt tot een vermindering van de BHR bij allergische CARA-patiënten.

Dit effect is niet afhankelijk van een gelijktijdig optredende verbetering van de longfunctie. De afname in BHR is dosis-afhankelijk. Individuele parameters, zoals inititiele mate van BHR, aantal eosinofiele cellen in het perifeer bloed en het serum IgE gehalte hebben geen voorspellende waarde voor het therapie-effect. Dit houdt in dat men mag verwachten dat hetzelfde gunstige effect optreedt bij niet-allergische CARA-patiënten, hetgeen overeenkomt met de klinische ervaring.

5. Hoewel het effect van corticosteroiden op de BHR past in de huidige opvattingen over het belang van luchtweginflammatie als oorzakelijke factor voor BHR, gaf de behandeling met hoge dosering budesonide gedurende 8 weken een bescheiden verbeterd effect op de BHR. De vraag in welke mate inflammatie oorzakelijk bijdraagt aan BHR, moet beantwoord worden door verder onderzoek waarbij langduriger wordt behandeld.

Het mag worden verwacht dat aanvullend onderzoek naar het effect van medicamenten op de BHR resultaten zal kunnen verschaffen die van belang zijn voor de klinische praktijk, maar ook antwoord kan geven op vragen omtrent het mechanisme van BHR en het belang van BHR als centraal fenomeen van CARA.

LIST OF ABBREVIATIONS

Anova	Analysis of variance
AP	Alkaline phosphatase
ASM	Airway smooth muscle
AsT	Aspartate aminotransferase
BHR	Bronchial hyperresponsiveness
BDP	Beclomethason diproprionate
BR	Bronchial responsiveness
CL	Drug total body clearance
CNSLD	Chronic non-specific lung disease
COLD	Chronic obstructive lung disease
DSCG	Disodium chromoglycate
EAR	Early asthmatic reaction
FEV_1	Forced expiratory volume
FVC	Forced vital capacity
GCS	Glucocorticosteroid
K_{el}	Elimination constant
LAR	Late asthmatic reaction
LTB_4	Leukotriene B_4
LTC_4	Leukotriene C_4
Manova	Multivariate analysis of variance
MEFV	Maximal expiratory flow-volume (curve)
NANC	Non-adrenergic non-cholinergic
PAF	Platelet activating factor
PC_{20}	Provocative concentration producing a fall in FEV_1 of 20%
PDE	phosphodiesterase
PgD_2	Prostaglandin D_2
Raw	Airway resistance
SD	Standard deviation
sGaw	Specific airways conductance
SEM	Standard error of the mean
SO_2	Sulphur dioxide
$t_{1/2}$	Plasma drug half-life
V_{ss}	Volume of distribution during steady state
V_c	Volume of the central compartment
$\dot{V}_{E, max\ 25}$	Maximal expiratory flow at 25% remaining volume
$\dot{V}_{E, max\ 50}$	Maximal expiratory flow at 50% remaining volume
$\dot{V}_{E, max\ 75}$	Maximal expiratory flow at 75% remaining volume

NAWOORD

Het in dit proefschrift beschreven onderzoek werd verricht binnen de afdelingen longziekten (prof.dr.H.J.Sluiter, prof.dr.G.H.Koëter), allergologie (prof.dr.K.de Vries) en longfunctie (prof.dr.R.Peset Reig) van de kliniek voor inwendige geneeskunde (prof.dr.E.Mandema). Daarnaast werd samengewerkt met Pharma Bio Research te Assen (dr.J.H.G.Jonkman). Het onderzoek werd mogelijk gemaakt door een subsidie van Astra Pharmaceutica bv Nederland.

Graag wil ik iedereen die aan de totstandkoming van dit proefschrift heeft bijgedragen hartelijk danken. In de eerste plaats dank ik alle patiënten en proefpersonen die bereid waren mee te doen aan dit onderzoek. Velen hebben er dagen voor moeten vrijmaken. Anderen hielden thuis nauwgezet meetgegevens bij. Zonder hun medewerking was dit onderzoek niet mogelijk geweest.

Prof.dr.G.H.Koëter ben ik veel dank verschuldigd voor de aanzet tot dit onderzoek. Dankzij zijn optimisme en voortdurend inspirerende begeleiding kwam het ook tot een afronding. Aan onze gesprekken, zelfs als die er toe leidden dat aanvankelijke pennevruchten geheel moesten worden omgewerkt, denk ik met plezier terug.

Prof.dr.K.de Vries dank ik voor het vertrouwen en de ruimte die hij bood om dit onderzoek op zijn afdeling te verrichten. Zijn kritische begeleiding heeft ertoe bijgedragen dat er in dit onderzoek aansluiting gevonden werd bij vroegere onderzoekingslijnen.

Dr.Th.W.v.d.Mark dank ik voor de begeleiding van dit onderzoek. Hij droeg nieuwe ideeën aan als stagnatie dreigde en hij was een onmisbare spil bij de analyse van de onderzoeksresultaten. Ik denk terug aan een plezierige tijd vooral dankzij zijn humor en relativerend vermogen.

Prof.dr.H.J.Sluiter deed de eerste suggesties voor dit onderzoek en gaf de ruimte eraan verder te werken. In de eerste plaats wil ik hem danken voor mijn opleiding tot longarts. Veel leerde ik van zijn brede kennis van het zich steeds uitbreidende vakgebied.

Dr.J.H.G.Jonkman leverde een belangrijke bijdrage aan theorie en praktijk van het pharmacokinetisch onderzoek. Ik dank hem voor onze plezierige samenwerking.

De heer M.Boorsma dank ik voor onze goede samenwerking. De manier waarop hij zich in de klinische aspecten van het onderzoek verdiepte dwong respect af.

Talloze inhalatie-provocaties en andere onderzoeken werden door mevrouw J.Kukler met veel inzet verricht. Tevens hield ze nauwgezet de boeken bij. Zonder haar inspanningen was het onderzoek niet mogelijk geweest.

De mede-auteurs prof.dr.C.H.Gips, dr.P.E.de Jong, mevrouw M.Laseur, de heer O.E.de Noord en prof.dr.R.A.de Zeeuw dank ik voor hun waardevolle bijdragen en kritieken.

I thank the co-authors L.Borgström, O.Borgå, I.Ekman, G.Erikson for our fruitfull coöperation and for their valuable remarks.

Ik dank dr.H.F.Kauffman, dr.A.Löwenberg, dr.H.Meurs, dr.J.G.R.de Monchy en dr.D.S.Postma voor de stimulerende discussies.

Alle medewerkers van de de afdelingen longziekten, longfunctie en allergologie die een bijdrage hebben geleverd aan dit onderzoek wil ik hartelijk dank zeggen. Zonder anderen te kort te doen dank ik in het bijzonder L.van Eerden, M.Lenters, D.S.Marrink, M.Nakken, T.Rakers, A.A.Smidt, H.Lange en F.Weeke.

J.H.Bosma, U.Bosma, S.W.Gremmer, P.de Vries en T.J.Otto bewerkten de manuscripten. B.Schaalma en E.Muskiet verzamelden literatuurgegevens. F.G.Sluiter corrigeerde de Engelse tekst. D.Buiter en J.Duitsch verzorgden het tekenwerk.

A.H.Holtkamp en W.J.van der Boon dank ik voor hun bijdrage aan het pharmacokinetisch onderzoek.

Mijn collega's te Leeuwarden J.Spijker, A.T.Huges en H.R.Pasma dank ik voor de tijd en ruimte die ze me gunden dit werk af te maken.

Mijn ouders ben ik dankbaar voor de gelegenheid die zij mij gaven te gaan studeren.

Tenslotte dank ik Joke, Lemke en Jitske voor het geduld en de geestelijke ondersteuning in de afgelopen periode.